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(54) Title: PROMOTION OR INHIBITION OF ANGIOGENESIS AND CARDIOVASCULARIZATION

(57) Abstract: Compositions and methods are disclosed for stimulating or inhibiting angiogenesis and/or cardiovascularization in mammals, including humans. Pharmaceutical compositions are based on polypeptides or antagonists thereto that have been identified for one or more of these uses. Disorders that can be diagnosed, prevented, or treated by the compositions herein include trauma such as wounds, various cancers, and disorders of the vessels including atherosclerosis and cardiac hypertrophy. In addition, the present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention, and to methods for producing the polypeptides of the present invention.

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PROMOTION OR INHIBITION OF ANGIOGENESIS AND CARDIOVASCULARIZATION

Background of the Invention

5 Field of the Invention

The present invention relates to compositions and methods useful for promoting or inhibiting angiogenesis and/or cardiovascularization in mammals in need of such biological effect. This includes the diagnosis and treatment of cardiovascular disorders as well as oncological disorders.

10 Description of Background

A. Cardiac Disorders and Factors

Heart failure affects approximately five million Americans, and new cases of heart failure number about 400,000 each year. It is the single most frequent cause of hospitalization for people age 65 and older in the United States. Recent advances in the management of acute cardiac diseases, including acute myocardial infarction, are
15 resulting in an expanding patient population that will eventually develop chronic heart failure. From 1979 to 1995, hospitalizations for congestive heart failure (CHF) rose from 377,000 to 872,000 (a 130 percent increase) and CHF deaths increased 116 percent.

CHF is a syndrome characterized by left ventricular dysfunction, reduced exercise tolerance, impaired quality of life, and markedly shortened life expectancy. The sine qua non of heart failure is an inability of the heart
20 to pump blood at a rate sufficient to meet the metabolic needs of the body's tissues (in other words, there is insufficient cardiac output).

At least four major compensatory mechanisms are activated in the setting of heart failure to boost cardiac output, including peripheral vasoconstriction, increased heart rate, increased cardiac contractility, and increased plasma volume. These effects are mediated primarily by the sympathetic nervous system and the renin-angiotensin system. See, Eichhorn, American Journal of Medicine, 104: 163-169 (1998). Increased output from the
25 sympathetic nervous system increases vascular tone, heart rate, and contractility. Angiotensin II elevates blood pressure by 1) directly stimulating vascular smooth muscle contraction, 2) promoting plasma volume expansion by stimulating aldosterone and antidiuretic hormone secretion, 3) stimulating sympathetic-mediated vascular tone, and 4) catalyzing the degradation of bradykinin, which has vasodilatory and natriuretic activity. See, review by Brown
30 and Vaughan, Circulation, 97: 1411-1420 (1998). As noted below, angiotensin II may also have directly deleterious effects on the heart by promoting myocyte necrosis (impairing systolic function) and intracardiac fibrosis (impairing diastolic and in some cases systolic function). See, Weber, Circulation, 96: 4065-4082 (1998).

A consistent feature of congestive heart failure (CHF) is cardiac hypertrophy, an enlargement of the heart that is activated by both mechanical and hormonal stimuli and enables the heart to adapt to demands for increased
35 cardiac output. Morgan and Baker, Circulation, 83: 13-25 (1991). This hypertrophic response is frequently

associated with a variety of distinct pathological conditions such as hypertension, aortic stenosis, myocardial infarction, cardiomyopathy, valvular regurgitation, and intracardiac shunt, all of which result in chronic hemodynamic overload.

Hypertrophy is generally defined as an increase in size of an organ or structure independent of natural growth that does not involve tumor formation. Hypertrophy of the heart is due either to an increase in the mass of the individual cells (myocytes), or to an increase in the number of cells making up the tissue (hyperplasia), or both. While the enlargement of an embryonic heart is largely dependent on an increase in myocyte number (which continues until shortly after birth), post-natal cardiac myocytes lose their proliferative capacity. Further growth occurs through hypertrophy of the individual cells.

Adult myocyte hypertrophy is initially beneficial as a short term response to impaired cardiac function by permitting a decrease in the load on individual muscle fibers. With severe, long-standing overload, however, the hypertrophied cells begin to deteriorate and die. Katz, "Heart Failure", in: Katz A.M. ed., Physiology of the Heart (New York: Raven Press, 1992) pp. 638-668. Cardiac hypertrophy is a significant risk factor for both mortality and morbidity in the clinical course of heart failure. Katz, Trends Cardiovasc. Med., 5: 37-44 (1995). For further details of the causes and pathology of cardiac hypertrophy see, e.g., Heart Disease, A Textbook of Cardiovascular Medicine, Braunwald, E. ed. (W.B. Saunders Co., 1988), Chapter 14, "Pathophysiology of Heart Failure."

On a cellular level, the heart is composed of myocytes and surrounding support cells, generically called non-myocytes. While non-myocytes are primarily fibroblast/mesenchymal cells, they also include endothelial and smooth muscle cells. Indeed, although myocytes make up most of the adult myocardial mass, they represent only about 30% of the total cell numbers present in heart. In response to hormonal, physiological, hemodynamic, and pathological stimuli, adult ventricular muscle cells can adapt to increased workloads through the activation of a hypertrophic process. This response is characterized by an increase in myocyte cell size and contractile protein content of individual cardiac muscle cells, without concomitant cell division and activation of embryonic genes, including the gene for atrial natriuretic peptide (ANP). Chien *et al.*, FASEB J., 5: 3037-3046 (1991); Chien *et al.*, Annu. Rev. Physiol., 55: 77-95 (1993). An increment in myocardial mass as a result of an increase in myocyte size that is associated with an accumulation of interstitial collagen within the extracellular matrix and around intramyocardial coronary arteries has been described in left ventricular hypertrophy secondary to pressure overload in humans. Caspari *et al.*, Cardiovasc. Res., 11: 554-558 (1977); Schwarz *et al.*, Am. J. Cardiol., 42: 895-903 (1978); Hess *et al.*, Circulation, 63: 360-371 (1981); Pearlman *et al.*, Lab. Invest., 46: 158-164 (1982).

It has also been suggested that paracrine factors produced by non-myocyte supporting cells may additionally be involved in the development of cardiac hypertrophy, and various non-myocyte derived hypertrophic factors, such as, leukocyte inhibitory factor (LIF) and endothelin, have been identified. Metcalf, Growth Factors, 7: 169-173 (1992); Kurzrock *et al.*, Endocrine Reviews, 12: 208-217 (1991); Inoue *et al.*, Proc. Natl. Acad. Sci. USA, 86: 2863-2867 (1989); Yanagisawa and Masaki, Trends Pharm. Sci., 10: 374-378 (1989); U.S. Patent No. 5,573,762 (issued November 12, 1996). Further exemplary factors that have been identified as potential mediators of cardiac hypertrophy include cardiotrophin-1 (CT-1) (Pennica *et al.*, Proc. Nat. Acad. Sci. USA, 92: 1142-1146 (1995)), catecholamines, adrenocorticosteroids, angiotensin, and prostaglandins.

At present, the treatment of cardiac hypertrophy varies depending on the underlying cardiac disease.

Catecholamines, adrenocorticosteroids, angiotensin, prostaglandins, LIF, endothelin (including endothelin-1, -2, and -3 and big endothelin), and CT-1 are among the factors identified as potential mediators of hypertrophy. For example, beta-adrenergic receptor blocking drugs (beta-blockers, *e.g.*, propranolol, timolol, tertalolol, carteolol, nadolol, betaxolol, penbutolol, acetobutolol, atenolol, metoprolol, carvedilol, etc.) and verapamil have been used extensively in the treatment of hypertrophic cardiomyopathy. The beneficial effects of beta-blockers on symptoms (*e.g.*, chest pain) and exercise tolerance are largely due to a decrease in the heart rate with a consequent prolongation of diastole and increased passive ventricular filling. Thompson *et al.*, Br. Heart J., **44**: 488-98 (1980); Harrison *et al.*, Circulation, **29**: 84-98 (1964). Verapamil has been described to improve ventricular filling and probably reducing myocardial ischemia. Bonow *et al.*, Circulation, **72**: 853-64 (1985).

Nifedipine and diltiazem have also been used occasionally in the treatment of hypertrophic cardiomyopathy. Lorell *et al.*, Circulation, **65**: 499-507 (1982); Betocchi *et al.*, Am. J. Cardiol., **78**: 451-457 (1996). However, because of its potent vasodilating properties, nifedipine may be harmful, especially in patients with outflow obstruction. Disopyramide has been used to relieve symptoms by virtue of its negative inotropic properties. Pollick, N. Engl. J. Med., **307**: 997-999 (1982). In many patients, however, the initial benefits decrease with time. Wigle *et al.*, Circulation, **92**: 1680-1692 (1995). Antihypertensive drug therapy has been reported to have beneficial effects on cardiac hypertrophy associated with elevated blood pressure. Examples of drugs used in antihypertensive therapy, alone or in combination, are calcium antagonists, *e.g.*, nitrendipine; adrenergic receptor blocking agents, *e.g.*, those listed above; angiotensin converting enzyme (ACE) inhibitors such as quinapril, captopril, enalapril, ramipril, benazepril, fosinopril, and lisinopril; diuretics, *e.g.*, chlorothiazide, hydrochlorothiazide, hydroflumethazide, methylchlorothiazide, benzthiazide, dichlorphenamide, acetazolamide, and indapamide; and calcium channel blockers, *e.g.*, diltiazem, nifedipine, verapamil, and nicardipine.

For example, treatment of hypertension with diltiazem and captopril showed a decrease in left ventricular muscle mass, but the Doppler indices of diastolic function did not normalize. Szlachcic *et al.*, Am. J. Cardiol., **63**: 198-201 (1989); Shahi *et al.*, Lancet, **336**: 458-461 (1990). These findings were interpreted to indicate that excessive amounts of interstitial collagen may remain after regression of left ventricular hypertrophy. Rossi *et al.*, Am. Heart J., **124**: 700-709 (1992). Rossi *et al.*, *supra*, investigated the effect of captopril on the prevention and regression of myocardial cell hypertrophy and interstitial fibrosis in pressure overload cardiac hypertrophy, in experimental rats.

Agents that increase cardiac contractility directly (inotropic agents) were initially thought to benefit patients with heart failure because they improved cardiac output in the short term. However, all positive inotropic agents except digoxigenin have been found to result in increased long-term mortality, in spite of short-term improvements in cardiac performance. Massie, Curr. Op. in Cardiology, **12**: 209-217 (1997); Reddy *et al.*, Curr. Opin. Cardiol., **12**: 233-241 (1997). Beta-adrenergic receptor blockers have recently been advocated for use in heart failure. Evidence from clinical trials suggests that improvements in cardiac function can be achieved without increased mortality, though documented improvements patient survival have not yet been demonstrated. See also, U.S. Pat. Nos. 5,935,924, 5,624,806; 5,661,122; and 5,610,134 and WO 95/28173 regarding the use of cardiotropin-I or antagonists thereof, or growth hormone and/or insulin-like growth factor-I in the treatment of CHF. Another treatment modality is heart transplantation, but this is limited by the availability of donor hearts.

Endothelin is a vasoconstricting peptide comprising 21 amino acids, isolated from swine arterial endothelial culture supernatant and structurally determined. Yanagisawa *et al.*, Nature, 332: 411-415 (1988). Endothelin was later found to exhibit various actions, and endothelin antibodies as endothelin antagonists have proven effective in the treatment of myocardial infarction, renal failure, and other diseases. Since endothelin is present in live bodies and exhibits vasoconstricting action, it is expected to be an endogenous factor involved in the regulation of the circulatory system, and may be associated with hypertension. cardiovascular diseases such as myocardial infarction, and renal diseases such as acute renal failure. Endothelin antagonists are described, for example, in U.S. Pat. No. 5,773,414; JP Pat. Publ. 3130299/1991, EP 457,195; EP 460,679; and EP 552,489. A new endothelin B receptor for identifying endothelin receptor antagonists is described in U.S. Pat. No. 5,773,223.

Current therapy for heart failure is primarily directed to using angiotensin-converting enzyme (ACE) inhibitors, such as captopril, and diuretics. These drugs improve hemodynamic profile and exercise tolerance and reduce the incidence of morbidity and mortality in patients with CHF. Kramer *et al.*, Circulation, 67(4): 807-816 (1983); Captopril Multicenter Research Group, J.A.C.C., 2(4): 755-763 (1983); The CONSENSUS Trial Study Group, N. Engl. J. Med., 316(23): 1429-1435 (1987); The SOLVD Investigators, N. Engl. J. Med., 325(5): 293-302 (1991). Further, they are useful in treating hypertension, left ventricular dysfunction, atherosclerotic vascular disease, and diabetic nephropathy. Brown and Vaughan, *supra*. However, despite proven efficacy, response to ACE inhibitors has been limited. For example, while prolonging survival in the setting of heart failure, ACE inhibitors appear to slow the progression towards end-stage heart failure, and substantial numbers of patients on ACE inhibitors have functional class III heart failure.

Moreover, improvement of functional capacity and exercise time is only small and mortality, although reduced, continues to be high. The CONSENSUS Trial Study Group, N. Engl. J. Med., 316(23): 1429-1453 (1987); The SOLVD Investigators, N. Engl. J. Med., 325(5): 293-302 (1991); Cohn *et al.*, N. Engl. J. Med., 325(5): 303-310 (1991); The Captopril-Digoxin Multicenter Research Group, JAMA, 259(4): 539-544 (1988). Hence, ACE inhibitors consistently appear unable to relieve symptoms in more than 60% of heart failure patients and reduce mortality of heart failure only by approximately 15-20%. For further adverse effects, *see* Brown and Vaughan, *supra*.

An alternative to ACE inhibitors is represented by specific AT1 receptor antagonists. Clinical studies are planned to compare the efficacy of these two modalities in the treatment of cardiovascular and renal disease. However, animal model data suggests that the ACE/Ang II pathway, while clearly involved in cardiac hypertrophy, is not the only, or even the primary pathway active in this role. Mouse genetic "knockout" models have been made to test individual components of the pathway. In one such model, the primary cardiac receptor for Ang II, AT sub 1A, has been genetically deleted; these mice do not develop hypertrophy when Ang II is given experimentally (confirming the basic success of the model in eliminating hypertrophy secondary to Ang II). However, when the aorta is constricted in these animals (a model of hypertensive cardiac stress), the hearts still become hypertrophic. This suggests that alternative signaling pathways, not depending on this receptor (AT sub 1A), are activated in hypertension. ACE inhibitors would presumably not be able to inhibit these pathways. *See*, Harada *et al.*, Circulation, 97: 1952-1959 (1998). *See also*, Homcy, Circulation, 97: 1890-1892 (1998) regarding the enigma associated with the process and mechanism of cardiac hypertrophy.

About 750,000 patients suffer from acute myocardial infarction (AMI) annually, and approximately one-fourth of all deaths in the United States are due to AMI. In recent years, thrombolytic agents, e.g., streptokinase, urokinase, and in particular tissue plasminogen activator (t-PA) have significantly increased the survival of patients who suffered myocardial infarction. When administered as a continuous intravenous infusion over 1.5 to 4 hours, t-PA produces coronary patency at 90 minutes in 69% to 90% of the treated patients. Topol *et al.*, Am. J. Cardiol., **61**: 723-728 (1988); Neuhaus *et al.*, J. Am. Coll. Cardiol., **12**: 581-587 (1988); Neuhaus *et al.*, J. Am. Coll. Cardiol., **14**: 1566-1569 (1989). The highest patency rates have been reported with high dose or accelerated dosing regimens. Topol, J. Am. Coll. Cardiol., **15**: 922-924 (1990). t-PA may also be administered as a single bolus, although due to its relatively short half-life, it is better suited for infusion therapy. Tebbe *et al.*, Am. J. Cardiol., **64**: 448-453 (1989). A t-PA variant, specifically designed to have longer half-life and very high fibrin specificity, TNK t-PA (a T103N, N117Q, KHRR(296-299)AAAA t-PA variant, Keyt *et al.*, Proc. Natl. Acad. Sci. USA, **91**: 3670-3674 (1994)) is particularly suitable for bolus administration. However, despite all these advances, the long-term prognosis of patient survival depends greatly on the post-infarction monitoring and treatment of the patients, which should include monitoring and treatment of cardiac hypertrophy.

B. Growth Factors

Various naturally occurring polypeptides reportedly induce the proliferation of endothelial cells. Among those polypeptides are the basic and acidic fibroblast growth factors (FGF) (Burgess and Maciag, Annual Rev. Biochem., **58**: 575 (1989)), platelet-derived endothelial cell growth factor (PD-ECGF) (Ishikawa *et al.*, Nature, **338**: 557 (1989)), and vascular endothelial growth factor (VEGF). Leung *et al.*, Science, **246**: 1306 (1989); Ferrara and Henzel, Biochem. Biophys. Res. Commun., **161**: 851 (1989); Tischer *et al.*, Biochem. Biophys. Res. Commun., **165**: 1198 (1989); EP 471,754B granted July 31, 1996.

Media conditioned by cells transfected with the human VEGF (hVEGF) cDNA promoted the proliferation of capillary endothelial cells, whereas control cells did not. Leung *et al.*, Science, **246**: 1306 (1989). Several additional cDNAs were identified in human cDNA libraries that encode 121-, 189-, and 206-amino acid isoforms of hVEGF (also collectively referred to as hVEGF-related proteins). The 121-amino acid protein differs from hVEGF by virtue of the deletion of the 44 amino acids between residues 116 and 159 in hVEGF. The 189-amino acid protein differs from hVEGF by virtue of the insertion of 24 amino acids at residue 116 in hVEGF, and apparently is identical to human vascular permeability factor (hVPF). The 206-amino acid protein differs from hVEGF by virtue of an insertion of 41 amino acids at residue 116 in hVEGF. Houck *et al.*, Mol. Endocrin., **5**: 1806 (1991); Ferrara *et al.*, J. Cell. Biochem., **47**: 211 (1991); Ferrara *et al.*, Endocrine Reviews, **13**: 18 (1992); Keck *et al.*, Science, **246**: 1309 (1989); Connolly *et al.*, J. Biol. Chem., **264**: 20017 (1989); EP 370,989 published May 30, 1990.

It is now well established that angiogenesis, which involves the formation of new blood vessels from preexisting endothelium, is implicated in the pathogenesis of a variety of disorders. These include solid tumors and metastasis, atherosclerosis, retrolental fibroplasia, hemangiomas, chronic inflammation, intraocular neovascular syndromes such as proliferative retinopathies, e.g., diabetic retinopathy, age-related macular degeneration (AMD), neovascular glaucoma, immune rejection of transplanted corneal tissue and other tissues, rheumatoid arthritis, and

psoriasis. Folkman *et al.*, J. Biol. Chem., **267**: 10931-10934 (1992); Klagsbrun *et al.*, Annu. Rev. Physiol., **53**: 217-239 (1991); and Garner A., "Vascular diseases". In: Pathobiology of Ocular Disease. A Dynamic Approach, Garner A., Klintworth GK, eds., 2nd Edition (Marcel Dekker, NY, 1994), pp 1625-1710.

In the case of tumor growth, angiogenesis appears to be crucial for the transition from hyperplasia to neoplasia, and for providing nourishment to the growing solid tumor. Folkman *et al.*, Nature, **339**: 58 (1989). The neovascularization allows the tumor cells to acquire a growth advantage and proliferative autonomy compared to the normal cells. Accordingly, a correlation has been observed between density of microvessels in tumor sections and patient survival in breast cancer as well as in several other tumors. Weidner *et al.*, N. Engl. J. Med., **324**: 1-6 (1991); Horak *et al.*, Lancet, **340**: 1120-1124 (1992); Macchiarini *et al.*, Lancet, **340**: 145-146 (1992).

The search for positive regulators of angiogenesis has yielded many candidates, including aFGF, bFGF, TGF- α , TGF- β , HGF, TNF- α , angiogenin, IL-8, etc. Folkman *et al.*, J.B.C., *supra*, and Klagsbrun *et al.*, *supra*. The negative regulators so far identified include thrombospondin (Good *et al.*, Proc. Natl. Acad. Sci. USA, **87**: 6624-6628 (1990)), the 16-kilodalton N-terminal fragment of prolactin (Clapp *et al.*, Endocrinology, **133**: 1292-1299 (1993)), angiostatin (O'Reilly *et al.*, Cell, **79**: 315-328 (1994)), and endostatin. O'Reilly *et al.*, Cell, **88**: 277-285 (1996).

Work done over the last several years has established the key role of VEGF, not only in stimulating vascular endothelial cell proliferation, but also in inducing vascular permeability and angiogenesis. Ferrara *et al.*, Endocr. Rev., **18**: 4-25 (1997). The finding that the loss of even a single VEGF allele results in embryonic lethality points to an irreplaceable role played by this factor in the development and differentiation of the vascular system. Furthermore, VEGF has been shown to be a key mediator of neovascularization associated with tumors and intraocular disorders. Ferrara *et al.*, Endocr. Rev., *supra*. The VEGF mRNA is overexpressed by the majority of human tumors examined. Berkman *et al.*, J. Clin. Invest., **91**: 153-159 (1993); Brown *et al.*, Human Pathol., **26**: 86-91 (1995); Brown *et al.*, Cancer Res., **53**: 4727-4735 (1993); Mattern *et al.*, Brit. J. Cancer, **73**: 931-934 (1996); Dvorak *et al.*, Am. J. Pathol., **146**: 1029-1039 (1995).

Also, the concentration levels of VEGF in eye fluids are highly correlated to the presence of active proliferation of blood vessels in patients with diabetic and other ischemia-related retinopathies. Aiello *et al.*, N. Engl. J. Med., **331**: 1480-1487 (1994). Furthermore, recent studies have demonstrated the localization of VEGF in choroidal neovascular membranes in patients affected by AMD. Lopez *et al.*, Invest. Ophthalmol. Vis. Sci., **37**: 855-868 (1996).

Anti-VEGF neutralizing antibodies suppress the growth of a variety of human tumor cell lines in nude mice (Kim *et al.*, Nature, **362**: 841-844 (1993); Warren *et al.*, J. Clin. Invest., **95**: 1789-1797 (1995); Borgström *et al.*, Cancer Res., **56**: 4032-4039 (1996); Melnyk *et al.*, Cancer Res., **56**: 921-924 (1996)) and also inhibit intraocular angiogenesis in models of ischemic retinal disorders. Adamis *et al.*, Arch. Ophthalmol., **114**: 66-71 (1996). Therefore, anti-VEGF monoclonal antibodies or other inhibitors of VEGF action are promising candidates for the treatment of solid tumors and various intraocular neovascular disorders. Such antibodies are described, for example, in EP 817,648 published January 14, 1998 and in WO98/45331 and WO98/45332 both published October 15, 1998.

There exist several other growth factors and mitogens, including transforming oncogenes, that are capable of

rapidly inducing a complex set of genes to be expressed by certain cells. Lau and Nathans, Molecular Aspects of Cellular Regulation, 6: 165-202 (1991). These genes, which have been named immediate-early- or early-response genes, are transcriptionally activated within minutes after contact with a growth factor or mitogen, independent of *de novo* protein synthesis. A group of these intermediate-early genes encodes secreted, extracellular proteins that are needed for coordination of complex biological processes such as differentiation and proliferation, regeneration, and wound healing. Ryseck *et al.*, Cell Growth Differ., 2: 235-233 (1991).

Highly-related proteins that belong to this group include *cef 10* (Simmons *et al.*, Proc. Natl. Acad. Sci. USA, 86: 1178-1182 (1989)), *cyr 61*, which is rapidly activated by serum- or platelet-derived growth factor (PDGF) (O'Brien *et al.*, Mol. Cell Biol., 10: 3569-3577 (1990), human connective tissue growth factor (CTGF) (Bradham *et al.*, J. Cell. Biol., 114: 1285-1294 (1991)), which is secreted by human vascular endothelial cells in high levels after activation with transforming growth factor beta (TGF- β), exhibits PDGF-like biological and immunological activities, and competes with PDGF for a particular cell surface receptor, *fisp-12* (Ryseck *et al.*, Cell Growth Differ., 2: 235-233 (1991)), human vascular IBP-like growth factor (VIGF) (WO 96/17931), and *nov*, normally arrested in adult kidney cells, which was found to be overexpressed in myeloblastosis-associated-virus-type-1-induced nephroblastomas. Jolait *et al.*, Mol. Cell. Biol., 12: 10-21 (1992).

The expression of these immediate-early genes acts as "third messengers" in the cascade of events triggered by growth factors. It is also thought that they are needed to integrate and coordinate complex biological processes, such as differentiation and wound healing in which cell proliferation is a common event.

As additional mitogens, insulin-like growth factor binding proteins (IGFBPs) have been shown, in complex with insulin-like growth factor (IGF), to stimulate increased binding of IGF to fibroblast and smooth muscle cell surface receptors. Clemmons *et al.*, J. Clin. Invest., 77: 1548 (1986). Inhibitory effects of IGFBP on various IGF actions *in vitro* include stimulation of glucose transport by adipocytes, sulfate incorporation by chondrocytes, and thymidine incorporation in fibroblast. Zapf *et al.*, J. Clin. Invest., 63: 1077 (1979). In addition, inhibitory effects of IGFBPs on growth factor-mediated mitogen activity in normal cells have been shown.

C. Need for Further Treatments

In view of the role of vascular endothelial cell growth and angiogenesis in many diseases and disorders, it is desirable to have a means of reducing or inhibiting one or more of the biological effects causing these processes. It is also desirable to have a means of assaying for the presence of pathogenic polypeptides in normal and diseased conditions, and especially cancer. Further, in a specific aspect, as there is no generally applicable therapy for the treatment of cardiac hypertrophy, the identification of factors that can prevent or reduce cardiac myocyte hypertrophy is of primary importance in the development of new therapeutic strategies to inhibit pathophysiological cardiac growth. While there are several treatment modalities for various cardiovascular and oncologic disorders, there is still a need for additional therapeutic approaches.

Summary of the Invention

A. Embodiments

Accordingly, the present invention concerns compositions and methods for promoting or inhibiting

angiogenesis and/or cardiovascularization in mammals. The present invention is based on the identification of proteins that test positive in various cardiovascular assays that test promotion or inhibition of certain biological activities. Accordingly, the proteins are believed to be useful drugs for the diagnosis and/or treatment (including prevention) of disorders where such effects are desired, such as the promotion or inhibition of angiogenesis, inhibition or stimulation of vascular endothelial cell growth, stimulation of growth or proliferation of vascular endothelial cells, inhibition of tumor growth, inhibition of angiogenesis-dependent tissue growth, stimulation of angiogenesis-dependent tissue growth, inhibition of cardiac hypertrophy and stimulation of cardiac hypertrophy, *e.g.*, for the treatment of congestive heart failure.

In one embodiment, the present invention provides a composition comprising a PRO polypeptide in admixture with a pharmaceutically acceptable carrier. In one aspect, the composition comprises a therapeutically effective amount of the polypeptide. In another aspect, the composition comprises a further active ingredient, namely, a cardiovascular, endothelial or angiogenic agent or an angiostatic agent, preferably an angiogenic or angiostatic agent. Preferably, the composition is sterile. The PRO polypeptide may be administered in the form of a liquid pharmaceutical formulation, which may be preserved to achieve extended storage stability. Preserved liquid pharmaceutical formulations might contain multiple doses of PRO polypeptide, and might, therefore, be suitable for repeated use.

In a further embodiment, the present invention provides a method for preparing such a composition useful for the treatment of a cardiovascular, endothelial or angiogenic disorder comprising admixing a therapeutically effective amount of a PRO polypeptide with a pharmaceutically acceptable carrier.

In another embodiment, the present invention provides a composition comprising an agonist or antagonist of a PRO polypeptide in admixture with a pharmaceutically acceptable carrier. In one aspect, the composition comprises a therapeutically effective amount of the agonist or antagonist. In another aspect, the composition comprises a further active ingredient, namely, a cardiovascular, endothelial or angiogenic agent or an angiostatic agent, preferably an angiogenic or angiostatic agent. Preferably, the composition is sterile. The PRO polypeptide agonist or antagonist may be administered in the form of a liquid pharmaceutical formulation, which may be preserved to achieve extended storage stability. Preserved liquid pharmaceutical formulations might contain multiple doses of a PRO polypeptide agonist or antagonist, and might, therefore, be suitable for repeated use.

In a further embodiment, the present invention provides a method for preparing such a composition useful for the treatment of a cardiovascular, endothelial or angiogenic disorder comprising admixing a therapeutically effective amount of a PRO polypeptide or agonist or antagonist with a pharmaceutically acceptable carrier.

In yet another embodiment, the present invention concerns a composition comprising an anti-PRO antibody in admixture with a pharmaceutically acceptable carrier. In one aspect, the composition comprises a therapeutically effective amount of the antibody. In another aspect, the composition comprises a further active ingredient, namely, a cardiovascular, endothelial or angiogenic agent or an angiostatic agent, preferably an angiogenic or angiostatic agent. Preferably, the composition is sterile. The composition may be administered in the form of a liquid pharmaceutical formulation, which may be preserved to achieve extended storage stability. Preserved liquid pharmaceutical formulations might contain multiple doses of the anti-PRO antibody, and might, therefore, be suitable for repeated use. In preferred embodiments, the antibody is a monoclonal antibody, an antibody fragment,

a humanized antibody, or a single-chain antibody.

In a further embodiment, the present invention provides a method for preparing such a composition useful for the treatment of a cardiovascular, endothelial or angiogenic disorder comprising admixing a therapeutically effective amount of an anti-PRO antibody with a pharmaceutically acceptable carrier.

5 In a still further aspect, the present invention provides an article of manufacture comprising:

(a) a composition of matter comprising a PRO polypeptide or agonist or antagonist thereof;

(b) a container containing said composition; and

(c) a label affixed to said container, or a package insert included in said container referring to the use of said PRO polypeptide or agonist or antagonist thereof in the treatment of a cardiovascular, endothelial or angiogenic disorder, wherein the agonist or antagonist may be an antibody which binds to the PRO polypeptide. The composition may comprise a therapeutically effective amount of the PRO polypeptide or the agonist or antagonist thereof.

In another embodiment, the present invention provides a method for identifying an agonist of a PRO polypeptide comprising:

15 (a) contacting cells and a test compound to be screened under conditions suitable for the induction of a cellular response normally induced by a PRO polypeptide; and

(b) determining the induction of said cellular response to determine if the test compound is an effective agonist, wherein the induction of said cellular response is indicative of said test compound being an effective agonist.

20 In another embodiment, the present invention provides a method for identifying an agonist of a PRO polypeptide comprising:

(a) contacting cells and a test compound to be screened under conditions suitable for the stimulation of cell proliferation by a PRO polypeptide; and

25 (b) measuring the proliferation of said cells to determine if the test compound is an effective agonist, wherein the stimulation of cell proliferation is indicative of said test compound being an effective agonist.

In another embodiment, the invention provides a method for identifying a compound that inhibits the activity of a PRO polypeptide comprising contacting a test compound with a PRO polypeptide under conditions and for a time sufficient to allow the test compound and polypeptide to interact and determining whether the activity of the PRO polypeptide is inhibited. In a specific preferred aspect, either the test compound or the PRO polypeptide is immobilized on a solid support. In another preferred aspect, the non-immobilized component carries a detectable label. In a preferred aspect, this method comprises the steps of:

30 (a) contacting cells and a test compound to be screened in the presence of a PRO polypeptide under conditions suitable for the induction of a cellular response normally induced by a PRO polypeptide; and

35 (b) determining the induction of said cellular response to determine if the test compound is an effective antagonist.

In another preferred aspect, this process comprises the steps of:

(a) contacting cells and a test compound to be screened in the presence of a PRO polypeptide under conditions suitable for the stimulation of cell proliferation by a PRO polypeptide; and

(b) measuring the proliferation of the cells to determine if the test compound is an effective antagonist.

In another embodiment, the invention provides a method for identifying a compound that inhibits the expression of a PRO polypeptide in cells that normally expresses the polypeptide, wherein the method comprises contacting the cells with a test compound and determining whether the expression of the PRO polypeptide is inhibited. In a preferred aspect, this method comprises the steps of:

(a) contacting cells and a test compound to be screened under conditions suitable for allowing expression of the PRO polypeptide; and

(b) determining the inhibition of expression of said polypeptide.

In a still further embodiment, the invention provides a compound that inhibits the expression of a PRO polypeptide, such as a compound that is identified by the methods set forth above.

Another aspect of the present invention is directed to an agonist or an antagonist of a PRO polypeptide which may optionally be identified by the methods described above.

One type of antagonist of a PRO polypeptide that inhibits one or more of the functions or activities of the PRO polypeptide is an antibody. Hence, in another aspect, the invention provides an isolated antibody that binds a PRO polypeptide. In a preferred aspect, the antibody is a monoclonal antibody, which preferably has non-human complementarity-determining-region (CDR) residues and human framework-region (FR) residues. The antibody may be labeled and may be immobilized on a solid support. In a further aspect, the antibody is an antibody fragment, a single-chain antibody, or a humanized antibody. Preferably, the antibody specifically binds to the polypeptide.

In a still further aspect, the present invention provides a method for diagnosing a disease or susceptibility to a disease which is related to a mutation in a PRO polypeptide-encoding nucleic acid sequence comprising determining the presence or absence of said mutation in the PRO polypeptide nucleic acid sequence, wherein the presence or absence of said mutation is indicative of the presence of said disease or susceptibility to said disease.

In a still further aspect, the invention provides a method of diagnosing a cardiovascular, endothelial or angiogenic disorder in a mammal which comprises analyzing the level of expression of a gene encoding a PRO polypeptide (a) in a test sample of tissue cells obtained from said mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower expression level in the test sample as compared to the control sample is indicative of the presence of a cardiovascular, endothelial or angiogenic disorder in said mammal. The expression of a gene encoding a PRO polypeptide may optionally be accomplished by measuring the level of mRNA or the polypeptide in the test sample as compared to the control sample.

In a still further aspect, the present invention provides a method of diagnosing a cardiovascular, endothelial or angiogenic disorder in a mammal which comprises detecting the presence or absence of a PRO polypeptide in a test sample of tissue cells obtained from said mammal, wherein the presence or absence of said PRO polypeptide in said test sample is indicative of the presence of a cardiovascular, endothelial or angiogenic disorder in said mammal.

In a still further embodiment, the invention provides a method of diagnosing a cardiovascular, endothelial or angiogenic disorder in a mammal comprising (a) contacting an anti-PRO antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the antibody and the PRO

polypeptide in the test sample, wherein the formation of said complex is indicative of the presence of a cardiovascular, endothelial or angiogenic disorder in the mammal. The detection may be qualitative or quantitative, and may be performed in comparison with monitoring the complex formation in a control sample of known normal tissue cells of the same cell type. A larger or smaller quantity of complexes formed in the test sample indicates the presence of a cardiovascular, endothelial or angiogenic dysfunction in the mammal from which the test tissue cells were obtained. The antibody preferably carries a detectable label. Complex formation can be monitored, for example, by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. The test sample is usually obtained from an individual suspected to have a cardiovascular, endothelial or angiogenic disorder.

In another embodiment, the invention provides a method for determining the presence of a PRO polypeptide in a sample comprising exposing a sample suspected of containing the PRO polypeptide to an anti-PRO antibody and determining binding of said antibody to a component of said sample. In a specific aspect, the sample comprises a cell suspected of containing the PRO polypeptide and the antibody binds to the cell. The antibody is preferably detectably labeled and/or bound to a solid support.

In further aspects, the invention provides a cardiovascular, endothelial or angiogenic disorder diagnostic kit comprising an anti-PRO antibody and a carrier in suitable packaging. Preferably, such kit further comprises instructions for using said antibody to detect the presence of the PRO polypeptide. Preferably, the carrier is a buffer, for example. Preferably, the cardiovascular, endothelial or angiogenic disorder is cancer.

In yet another embodiment, the present invention provides a method for treating a cardiovascular, endothelial or angiogenic disorder in a mammal comprising administering to the mammal an effective amount of a PRO polypeptide. Preferably, the disorder is cardiac hypertrophy, trauma such as wounds or burns, or a type of cancer. In a further aspect, the mammal is further exposed to angioplasty or a drug that treats cardiovascular, endothelial or angiogenic disorders such as ACE inhibitors or chemotherapeutic agents if the cardiovascular, endothelial or angiogenic disorder is a type of cancer. Preferably, the mammal is human, preferably one who is at risk of developing cardiac hypertrophy and more preferably has suffered myocardial infarction.

In another preferred aspect, the cardiac hypertrophy is characterized by the presence of an elevated level of $\text{PGF}_{2\alpha}$. Alternatively, the cardiac hypertrophy may be induced by myocardial infarction, wherein preferably the administration of the PRO polypeptide is initiated within 48 hours, more preferably within 24 hours, following myocardial infarction.

In another preferred embodiment, the cardiovascular, endothelial or angiogenic disorder is cardiac hypertrophy and said PRO polypeptide is administered together with a cardiovascular, endothelial or angiogenic agent. The preferred cardiovascular, endothelial or angiogenic agent for this purpose is selected from the group consisting of an antihypertensive drug, an ACE inhibitor, an endothelin receptor antagonist and a thrombolytic agent. If a thrombolytic agent is administered, preferably the PRO polypeptide is administered following administration of such agent. More preferably, the thrombolytic agent is recombinant human tissue plasminogen activator.

In another preferred aspect, the cardiovascular, endothelial or angiogenic disorder is cardiac hypertrophy and the PRO polypeptide is administered following primary angioplasty for the treatment of acute myocardial infarction, preferably wherein the mammal is further exposed to angioplasty or a cardiovascular, endothelial, or angiogenic agent.

In another preferred embodiment, the cardiovascular, endothelial or angiogenic disorder is a cancer and the PRO polypeptide is administered in combination with a chemotherapeutic agent, a growth inhibitory agent or a cytotoxic agent.

5 In a further embodiment, the invention concerns a method for treating a cardiovascular, endothelial or angiogenic disorder in a mammal comprising administering to the mammal an effective amount of an agonist of a PRO polypeptide. Preferably, the cardiovascular, endothelial or angiogenic disorder is cardiac hypertrophy, trauma, a cancer, or age-related macular degeneration. Also preferred is where the mammal is human, and where an effective amount of an angiogenic or angiostatic agent is administered in conjunction with the agonist.

10 In a further embodiment, the invention concerns a method for treating a cardiovascular, endothelial or angiogenic disorder in a mammal comprising administering to the mammal an effective amount of an antagonist of a PRO polypeptide. Preferably, the cardiovascular, endothelial or angiogenic disorder is cardiac hypertrophy, trauma, a cancer, or age-related macular degeneration. Also preferred is where the mammal is human, and where an effective amount of an angiogenic or angiostatic agent is administered in conjunction with the antagonist.

15 In a further embodiment, the invention concerns a method for treating a cardiovascular, endothelial or angiogenic disorder in a mammal comprising administering to the mammal an effective amount of an anti-PRO antibody. Preferably, the cardiovascular, endothelial or angiogenic disorder is cardiac hypertrophy, trauma, a cancer, or age-related macular degeneration. Also preferred is where the mammal is human, and where an effective amount of an angiogenic or angiostatic agent is administered in conjunction with the antibody.

20 In still further embodiments, the invention provides a method for treating a cardiovascular, endothelial or angiogenic disorder in a mammal that suffers therefrom comprising administering to the mammal a nucleic acid molecule that codes for either (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide or (c) an antagonist of a PRO polypeptide, wherein said agonist or antagonist may be an anti-PRO antibody. In a preferred embodiment, the mammal is human. In another preferred embodiment, the gene is administered via *ex vivo* gene therapy. In a further preferred embodiment, the gene is comprised within a vector, more preferably an adenoviral, 25 adeno-associated viral, lentiviral, or retroviral vector.

In yet another aspect, the invention provides a recombinant retroviral particle comprising a retroviral vector consisting essentially of a promoter, nucleic acid encoding (a) a PRO polypeptide, (b) an agonist polypeptide of a PRO polypeptide, or (c) an antagonist polypeptide of a PRO polypeptide, and a signal sequence for cellular secretion of the polypeptide, wherein the retroviral vector is in association with retroviral structural proteins. 30 Preferably, the signal sequence is from a mammal, such as from a native PRO polypeptide.

In a still further embodiment, the invention supplies an *ex vivo* producer cell comprising a nucleic acid construct that expresses retroviral structural proteins and also comprises a retroviral vector consisting essentially of a promoter, nucleic acid encoding (a) a PRO polypeptide, (b) an agonist polypeptide of a PRO polypeptide or (c) an antagonist polypeptide of a PRO polypeptide, and a signal sequence for cellular secretion of the polypeptide, 35 wherein said producer cell packages the retroviral vector in association with the structural proteins to produce recombinant retroviral particles.

In yet another embodiment, the invention provides a method for inhibiting endothelial cell growth in a mammal comprising administering to the mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an

antagonist of a PRO polypeptide, wherein endothelial cell growth in said mammal is inhibited, and wherein said agonist or antagonist may be an anti-PRO antibody. Preferably, the mammal is human and the endothelial cell growth is associated with a tumor or a retinal disorder.

5 In yet another embodiment, the invention provides a method for stimulating endothelial cell growth in a mammal comprising administering to the mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein endothelial cell growth in said mammal is stimulated, and wherein said agonist or antagonist may be an anti-PRO antibody. Preferably, the mammal is human.

10 In yet another embodiment, the invention provides a method for inhibiting cardiac hypertrophy in a mammal comprising administering to the mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein cardiac hypertrophy in said mammal is inhibited, and wherein said agonist or antagonist may be an anti-PRO antibody. Preferably, the mammal is human and the cardiac hypertrophy has been induced by myocardial infarction.

15 In yet another embodiment, the invention provides a method for stimulating cardiac hypertrophy in a mammal comprising administering to the mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein cardiac hypertrophy in said mammal is stimulated, and wherein said agonist or antagonist may be an anti-PRO antibody. Preferably, the mammal is human who suffers from congestive heart failure.

20 In yet another embodiment, the invention provides a method for inhibiting angiogenesis induced by a PRO polypeptide in a mammal comprising administering a therapeutically effective amount of an anti-PRO antibody to the mammal. Preferably, the mammal is a human, and more preferably the mammal has a tumor or a retinal disorder.

25 In yet another embodiment, the invention provides a method for stimulating angiogenesis induced by a PRO polypeptide in a mammal comprising administering a therapeutically effective amount of a PRO polypeptide to the mammal. Preferably, the mammal is a human, and more preferably angiogenesis would promote tissue regeneration or wound healing.

30 In yet another embodiment, the invention provides a method for inhibiting endothelial cell growth in a mammal comprising administering to the mammal a PRO175, PRO183, PRO198, PRO202, PRO364, PRO535, PRO819, PRO877, PRO879, PRO1002 or PRO1304 polypeptide or agonist thereof, wherein endothelial cell growth in said mammal is inhibited.

35 In yet another embodiment, the invention provides a method for stimulating endothelial cell growth in a mammal comprising administering to the mammal a PRO356, PRO861, PRO1308 or PRO1710 polypeptide or agonist thereof, wherein endothelial cell growth in said mammal is stimulated.

In yet another embodiment, the invention provides a method for inhibiting endothelial cell growth in a mammal comprising administering to the mammal an antagonist of a PRO356, PRO861, PRO1308 or PRO1710 polypeptide, wherein endothelial cell growth in said mammal is inhibited.

In yet another embodiment, the invention provides a method for stimulating endothelial cell growth in a mammal comprising administering to the mammal an antagonist of a PRO175, PRO183, PRO198, PRO202, PRO364, PRO535, PRO819, PRO877, PRO879, PRO1002 or PRO1304 polypeptide, wherein endothelial cell

growth in said mammal is stimulated.

In yet another embodiment, the invention provides a method for inducing cardiac hypertrophy in a mammal comprising administering to the mammal a PRO183, PRO200, PRO202, or PRO882 polypeptide or agonist thereof, wherein cardiac hypertrophy in said mammal is induced.

5 In yet another embodiment, the invention provides a method for reducing cardiac hypertrophy in a mammal comprising administering to the mammal an antagonist of a PRO183, PRO200, PRO202, or PRO882 polypeptide, wherein cardiac hypertrophy in said mammal is reduced.

10 In yet another embodiment, the invention provides a method for inhibiting angiogenesis induced by a PRO861 polypeptide comprising administering a therapeutically effective amount of an anti-PRO861 antibody to the mammal, wherein said angiogenesis is inhibited.

In yet another embodiment, the invention provides a method for stimulating angiogenesis induced by a PRO861 polypeptide comprising administering a therapeutically effective amount of said polypeptide to the mammal, wherein said angiogenesis is stimulated.

15 In yet another embodiment, the invention provides a method for inhibiting angiogenesis in a mammal comprising administering a therapeutically effective amount of PRO198, PRO877, PRO1002 or PRO1304 polypeptide to the mammal, wherein said angiogenesis is inhibited.

20 In yet another embodiment, the invention provides a method for stimulating angiogenesis in a mammal comprising administering a therapeutically effective amount of an anti-PRO198, anti-PRO877, anti-PRO1002 or anti-PRO1304 polypeptide to the mammal, wherein said angiogenesis is stimulated.

B. Additional Embodiments

In other embodiments of the present invention, the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence that encodes a PRO polypeptide.

25 In one aspect, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule encoding a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined

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fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

In other aspects, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule comprising the coding sequence of a full-length PRO polypeptide cDNA as disclosed herein, the coding sequence of a PRO polypeptide lacking the signal peptide as disclosed herein, the coding sequence of an extracellular domain of a transmembrane PRO polypeptide, with or without the signal peptide, as disclosed herein or the coding sequence of any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

In a further aspect, the invention concerns an isolated nucleic acid molecule comprising a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule that encodes the same mature polypeptide encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein, or (b) the complement of the DNA molecule of (a).

Another aspect of the present invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated, or is complementary to such encoding nucleotide sequence, wherein the transmembrane domain(s) of such polypeptide are disclosed herein. Therefore, soluble extracellular domains of the herein

described PRO polypeptides are contemplated.

Another embodiment is directed to fragments of a PRO polypeptide coding sequence, or the complement thereof, that may find use as, for example, hybridization probes, for encoding fragments of a PRO polypeptide that may optionally encode a polypeptide comprising a binding site for an anti-PRO antibody or as antisense oligonucleotide probes. Such nucleic acid fragments are usually at least about 20 nucleotides in length, alternatively at least about 30 nucleotides in length, alternatively at least about 40 nucleotides in length, alternatively at least about 50 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 70 nucleotides in length, alternatively at least about 80 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 100 nucleotides in length, alternatively at least about 110 nucleotides in length, alternatively at least about 120 nucleotides in length, alternatively at least about 130 nucleotides in length, alternatively at least about 140 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 160 nucleotides in length, alternatively at least about 170 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 190 nucleotides in length, alternatively at least about 200 nucleotides in length, alternatively at least about 250 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 350 nucleotides in length, alternatively at least about 400 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 500 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 700 nucleotides in length, alternatively at least about 800 nucleotides in length, alternatively at least about 900 nucleotides in length and alternatively at least about 1000 nucleotides in length, wherein in this context the term "about" means the referenced nucleotide sequence length plus or minus 10% of that referenced length. It is noted that novel fragments of a PRO polypeptide-encoding nucleotide sequence may be determined in a routine manner by aligning the PRO polypeptide-encoding nucleotide sequence with other known nucleotide sequences using any of a number of well known sequence alignment programs and determining which PRO polypeptide-encoding nucleotide sequence fragment(s) are novel. All of such PRO polypeptide-encoding nucleotide sequences are contemplated herein. Also contemplated are the PRO polypeptide fragments encoded by these nucleotide molecule fragments, preferably those PRO polypeptide fragments that comprise a binding site for an anti-PRO antibody.

In another embodiment, the invention provides an isolated PRO polypeptide encoded by any of the isolated nucleic acid sequences hereinabove identified.

In a certain aspect, the invention concerns an isolated PRO polypeptide, comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid

sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein.

In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to an amino acid sequence encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein.

In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid sequence scoring at least about 80% positives, alternatively at least about 81% positives, alternatively at least about 82% positives, alternatively at least about 83% positives, alternatively at least about 84% positives, alternatively at least about 85% positives, alternatively at least about 86% positives, alternatively at least about 87% positives, alternatively at least about 88% positives, alternatively at least about 89% positives, alternatively at least about 90% positives, alternatively at least about 91% positives, alternatively at least about 92% positives, alternatively at least about 93% positives, alternatively at least about 94% positives, alternatively at least about 95% positives, alternatively at least about 96% positives, alternatively at least about 97% positives, alternatively at least about 98% positives and alternatively at least about 99% positives when compared with the amino acid sequence of a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein.

In a specific aspect, the invention provides an isolated PRO polypeptide without the N-terminal signal sequence and/or the initiating methionine and is encoded by a nucleotide sequence that encodes such an amino acid sequence as hereinbefore described. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from

the cell culture.

Another aspect of the invention provides an isolated PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

In yet another embodiment, the invention concerns agonists and antagonists of a native PRO polypeptide as defined herein. In a particular embodiment, the agonist or antagonist is an anti-PRO antibody or a small molecule.

In a further embodiment, the invention concerns a method of identifying agonists or antagonists to a PRO polypeptide which comprise contacting the PRO polypeptide with a candidate molecule and monitoring a biological activity mediated by said PRO polypeptide. Preferably, the PRO polypeptide is a native PRO polypeptide.

In a still further embodiment, the invention concerns a composition of matter comprising a PRO polypeptide, or an agonist or antagonist of a PRO polypeptide as herein described, or an anti-PRO antibody, in combination with a carrier. Optionally, the carrier is a pharmaceutically acceptable carrier.

Another embodiment of the present invention is directed to the use of a PRO polypeptide, or an agonist or antagonist thereof as hereinbefore described, or an anti-PRO antibody, for the preparation of a medicament useful in the treatment of a condition which is responsive to the PRO polypeptide, an agonist or antagonist thereof or an anti-PRO antibody.

In additional embodiments of the present invention, the invention provides vectors comprising DNA encoding any of the herein described polypeptides. Host cell comprising any such vector are also provided. By way of example, the host cells may be CHO cells, *E. coli*, yeast, or Baculovirus-infected insect cells. A process for producing any of the herein described polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of the desired polypeptide and recovering the desired polypeptide from the cell culture.

In other embodiments, the invention provides chimeric molecules comprising any of the herein described polypeptides fused to a heterologous polypeptide or amino acid sequence. Example of such chimeric molecules comprise any of the herein described polypeptides fused to an epitope tag sequence or a Fc region of an immunoglobulin.

In yet another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody, humanized antibody, antibody fragment or single-chain antibody.

In yet other embodiments, the invention provides oligonucleotide probes useful for isolating genomic and cDNA nucleotide sequences or as antisense probes, wherein those probes may be derived from any of the above or below described nucleotide sequences.

Brief Description of the Drawings

Figure 1 shows a nucleotide sequence (SEQ ID NO:1) of a native sequence PRO175 cDNA, wherein SEQ ID NO:1 is a clone designated herein as "DNA19355-1150-1".

Figure 2 shows the amino acid sequence (SEQ ID NO:2) derived from the coding sequence of SEQ ID NO:1 shown in Figure 1.

Figure 3 shows a nucleotide sequence (SEQ ID NO:3) of a native sequence PRO200 cDNA, wherein SEQ ID NO:3 is a clone designated herein as "DNA29101-1122".

5 Figure 4 shows the amino acid sequence (SEQ ID NO:4) derived from the coding sequence of SEQ ID NO:3 shown in Figure 3.

Figure 5 shows a nucleotide sequence (SEQ ID NO:5) of a native sequence PRO198 cDNA, wherein SEQ ID NO:5 is a clone designated herein as "DNA33457-1078".

10 Figure 6 shows the amino acid sequence (SEQ ID NO:6) derived from the coding sequence of SEQ ID NO:5 shown in Figure 5.

Figure 7 shows a nucleotide sequence (SEQ ID NO:7) of a native sequence PRO364 cDNA, wherein SEQ ID NO:7 is a clone designated herein as "DNA47365-1206".

Figure 8 shows the amino acid sequence (SEQ ID NO:8) derived from the coding sequence of SEQ ID NO:7 shown in Figure 7.

15 Figure 9 shows a nucleotide sequence (SEQ ID NO:9) of a native sequence PRO356 cDNA, wherein SEQ ID NO:9 is a clone designated herein as "DNA47470-1130-P1".

Figure 10 shows the amino acid sequence (SEQ ID NO:10) derived from the coding sequence of SEQ ID NO:9 shown in Figure 9.

20 Figure 11 shows a nucleotide sequence (SEQ ID NO:11) of a native sequence PRO535 cDNA, wherein SEQ ID NO:11 is a clone designated herein as "DNA49143-1429".

Figure 12 shows the amino acid sequence (SEQ ID NO:12) derived from the coding sequence of SEQ ID NO:11 shown in Figure 11.

Figure 13 shows a nucleotide sequence (SEQ ID NO:13) of a native sequence PRO819 cDNA, wherein SEQ ID NO:13 is a clone designated herein as "DNA57695-1340".

25 Figure 14 shows the amino acid sequence (SEQ ID NO:14) derived from the coding sequence of SEQ ID NO:13 shown in Figure 13.

Figure 15 shows a nucleotide sequence (SEQ ID NO:15) of a native sequence PRO1002 cDNA, wherein SEQ ID NO:15 is a clone designated herein as "DNA59208-1373".

30 Figure 16 shows the amino acid sequence (SEQ ID NO:16) derived from the coding sequence of SEQ ID NO:15 shown in Figure 15.

Figure 17 shows a nucleotide sequence (SEQ ID NO:17) of a native sequence PRO1308 cDNA, wherein SEQ ID NO:17 is a clone designated herein as "DNA62306-1570".

Figure 18 shows the amino acid sequence (SEQ ID NO:18) derived from the coding sequence of SEQ ID NO:17 shown in Figure 17.

35 Figure 19 shows a nucleotide sequence (SEQ ID NO:19) of a native sequence PRO1304 cDNA, wherein SEQ ID NO:19 is a clone designated herein as "DNA65406-1567".

Figure 20 shows the amino acid sequence (SEQ ID NO:20) derived from the coding sequence of SEQ ID NO:19 shown in Figure 19.

Figure 21 shows a nucleotide sequence (SEQ ID NO:21) of a native sequence PRO183 cDNA, wherein SEQ ID NO:21 is a clone designated herein as "DNA28498".

Figure 22 shows the amino acid sequence (SEQ ID NO:22) derived from the coding sequence of SEQ ID NO:21 shown in Figure 21.

5 Figure 23 shows a nucleotide sequence (SEQ ID NO:23) of a native sequence PRO202 cDNA, wherein SEQ ID NO:23 is a clone designated herein as "DNA30869".

Figure 24 shows the amino acid sequence (SEQ ID NO:24) derived from the coding sequence of SEQ ID NO:23 shown in Figure 23.

10 Figure 25 shows a nucleotide sequence (SEQ ID NO:25) of a native sequence PRO861 cDNA, wherein SEQ ID NO:25 is a clone designated herein as "DNA50798".

Figure 26 shows the amino acid sequence (SEQ ID NO:26) derived from the coding sequence of SEQ ID NO:25 shown in Figure 25.

Figure 27 shows a nucleotide sequence (SEQ ID NO:27) of a native sequence PRO877 cDNA, wherein SEQ ID NO:27 is a clone designated herein as "DNA58120".

15 Figure 28 shows the amino acid sequence (SEQ ID NO:28) derived from the coding sequence of SEQ ID NO:27 shown in Figure 27.

Figure 29 shows a nucleotide sequence (SEQ ID NO:29) of a native sequence PRO879 cDNA, wherein SEQ ID NO:29 is a clone designated herein as "DNA58122".

20 Figure 30 shows the amino acid sequence (SEQ ID NO:30) derived from the coding sequence of SEQ ID NO:29 shown in Figure 29.

Figure 31 shows a nucleotide sequence (SEQ ID NO:31) of a native sequence PRO882 cDNA, wherein SEQ ID NO:31 is a clone designated herein as "DNA58125".

Figure 32 shows the amino acid sequence (SEQ ID NO:32) derived from the coding sequence of SEQ ID NO:31 shown in Figure 31.

25 Figure 33 shows a nucleotide sequence (SEQ ID NO:33) of a native sequence PRO1710 cDNA, wherein SEQ ID NO:33 is a clone designated herein as "DNA82331".

Figure 34 shows the amino acid sequence (SEQ ID NO:34) derived from the coding sequence of SEQ ID NO:33 shown in Figure 33.

30 Detailed Description of the Invention

I. Definitions

35 The phrases "cardiovascular, endothelial and angiogenic disorder", "cardiovascular, endothelial and angiogenic dysfunction", "cardiovascular, endothelial or angiogenic disorder" and "cardiovascular, endothelial or angiogenic dysfunction" are used interchangeably and refer in part to systemic disorders that affect vessels, such as diabetes mellitus, as well as diseases of the vessels themselves, such as of the arteries, capillaries, veins, and/or lymphatics. This would include indications that stimulate angiogenesis and/or cardiovascularization, and those that inhibit angiogenesis and/or cardiovascularization. Such disorders include, for example, arterial disease, such as atherosclerosis, hypertension, inflammatory vasculitides, Reynaud's disease and Reynaud's phenomenon, aneurysms,

and arterial restenosis; venous and lymphatic disorders such as thrombophlebitis, lymphangitis, and lymphedema; and other vascular disorders such as peripheral vascular disease, cancer such as vascular tumors. *e.g.*, hemangioma (capillary and cavernous), glomus tumors, telangiectasia, bacillary angiomatosis, hemangioendothelioma, angiosarcoma, haemangiopericytoma, Kaposi's sarcoma, lymphangioma, and lymphangiosarcoma, tumor angiogenesis, trauma such as wounds, burns, and other injured tissue, implant fixation, scarring, ischemia reperfusion injury, rheumatoid arthritis, cerebrovascular disease, renal diseases such as acute renal failure, and osteoporosis. This would also include angina, myocardial infarctions such as acute myocardial infarctions, cardiac hypertrophy, and heart failure such as CHF.

"Hypertrophy", as used herein, is defined as an increase in mass of an organ or structure independent of natural growth that does not involve tumor formation. Hypertrophy of an organ or tissue is due either to an increase in the mass of the individual cells (true hypertrophy), or to an increase in the number of cells making up the tissue (hyperplasia), or both. Certain organs, such as the heart, lose the ability to divide shortly after birth. Accordingly, "cardiac hypertrophy" is defined as an increase in mass of the heart, which, in adults, is characterized by an increase in myocyte cell size and contractile protein content without concomitant cell division. The character of the stress responsible for inciting the hypertrophy, (*e.g.*, increased preload, increased afterload, loss of myocytes, as in myocardial infarction, or primary depression of contractility), appears to play a critical role in determining the nature of the response. The early stage of cardiac hypertrophy is usually characterized morphologically by increases in the size of myofibrils and mitochondria, as well as by enlargement of mitochondria and nuclei. At this stage, while muscle cells are larger than normal, cellular organization is largely preserved. At a more advanced stage of cardiac hypertrophy, there are preferential increases in the size or number of specific organelles, such as mitochondria, and new contractile elements are added in localized areas of the cells, in an irregular manner. Cells subjected to long-standing hypertrophy show more obvious disruptions in cellular organization, including markedly enlarged nuclei with highly lobulated membranes, which displace adjacent myofibrils and cause breakdown of normal Z-band registration. The phrase "cardiac hypertrophy" is used to include all stages of the progression of this condition, characterized by various degrees of structural damage of the heart muscle, regardless of the underlying cardiac disorder. Hence, the term also includes physiological conditions instrumental in the development of cardiac hypertrophy, such as elevated blood pressure, aortic stenosis, or myocardial infarction.

"Heart failure" refers to an abnormality of cardiac function where the heart does not pump blood at the rate needed for the requirements of metabolizing tissues. The heart failure can be caused by a number of factors, including ischemic, congenital, rheumatic, or idiopathic forms.

"Congestive heart failure" (CHF) is a progressive pathologic state where the heart is increasingly unable to supply adequate cardiac output (the volume of blood pumped by the heart over time) to deliver the oxygenated blood to peripheral tissues. As CHF progresses, structural and hemodynamic damages occur. While these damages have a variety of manifestations, one characteristic symptom is ventricular hypertrophy. CHF is a common end result of a number of various cardiac disorders.

"Myocardial infarction" generally results from atherosclerosis of the coronary arteries, often with superimposed coronary thrombosis. It may be divided into two major types: transmural infarcts, in which myocardial necrosis involves the full thickness of the ventricular wall, and subendocardial (nontransmural) infarcts,

in which the necrosis involves the subendocardium, the intramural myocardium, or both, without extending all the way through the ventricular wall to the epicardium. Myocardial infarction is known to cause both a change in hemodynamic effects and an alteration in structure in the damaged and healthy zones of the heart. Thus, for example, myocardial infarction reduces the maximum cardiac output and the stroke volume of the heart. Also associated with myocardial infarction is a stimulation of the DNA synthesis occurring in the interstice as well as an increase in the formation of collagen in the areas of the heart not affected.

As a result of the increased stress or strain placed on the heart in prolonged hypertension due, for example, to the increased total peripheral resistance, cardiac hypertrophy has long been associated with "hypertension". A characteristic of the ventricle that becomes hypertrophic as a result of chronic pressure overload is an impaired diastolic performance. Fouad *et al.*, J. Am. Coll. Cardiol., 4: 1500-1506 (1984); Smith *et al.*, J. Am. Coll. Cardiol., 5: 869-874 (1985). A prolonged left ventricular relaxation has been detected in early essential hypertension, in spite of normal or supranormal systolic function. Hartford *et al.*, Hypertension, 6: 329-338 (1984). However, there is no close parallelism between blood pressure levels and cardiac hypertrophy. Although improvement in left ventricular function in response to antihypertensive therapy has been reported in humans, patients variously treated with a diuretic (hydrochlorothiazide), a β -blocker (propranolol), or a calcium channel blocker (diltiazem), have shown reversal of left ventricular hypertrophy, without improvement in diastolic function. Inouye *et al.*, Am. J. Cardiol., 53: 1583-7 (1984).

Another complex cardiac disease associated with cardiac hypertrophy is "hypertrophic cardiomyopathy". This condition is characterized by a great diversity of morphologic, functional, and clinical features (Maron *et al.*, N. Engl. J. Med., 316: 780-789 (1987); Spirito *et al.*, N. Engl. J. Med., 320: 749-755 (1989); Louie and Edwards, Prog. Cardiovasc. Dis., 36: 275-308 (1994); Wigle *et al.*, Circulation, 92: 1680-1692 (1995)), the heterogeneity of which is accentuated by the fact that it afflicts patients of all ages. Spirito *et al.*, N. Engl. J. Med., 336: 775-785 (1997). The causative factors of hypertrophic cardiomyopathy are also diverse and little understood. In general, mutations in genes encoding sarcomeric proteins are associated with hypertrophic cardiomyopathy. Recent data suggest that β -myosin heavy chain mutations may account for approximately 30 to 40 percent of cases of familial hypertrophic cardiomyopathy. Watkins *et al.*, N. Engl. J. Med., 326: 1108-1114 (1992); Schwartz *et al.*, Circulation, 91: 532-540 (1995); Marian and Roberts, Circulation, 92: 1336-1347 (1995); Thierfelder *et al.*, Cell, 77: 701-712 (1994); Watkins *et al.*, Nat. Gen., 11: 434-437 (1995). Besides β -myosin heavy chain, other locations of genetic mutations include cardiac troponin T, alpha topomyosin, cardiac myosin binding protein C, essential myosin light chain, and regulatory myosin light chain. See, Malik and Watkins, Curr. Opin. Cardiol., 12: 295-302 (1997).

Supravalvular "aortic stenosis" is an inherited vascular disorder characterized by narrowing of the ascending aorta, but other arteries, including the pulmonary arteries, may also be affected. Untreated aortic stenosis may lead to increased intracardiac pressure resulting in myocardial hypertrophy and eventually heart failure and death. The pathogenesis of this disorder is not fully understood, but hypertrophy and possibly hyperplasia of medial smooth muscle are prominent features of this disorder. It has been reported that molecular variants of the elastin gene are involved in the development and pathogenesis of aortic stenosis. U.S. Patent No. 5,650,282 issued July 22, 1997.

"Valvular regurgitation" occurs as a result of heart diseases resulting in disorders of the cardiac valves. Various diseases, like rheumatic fever, can cause the shrinking or pulling apart of the valve orifice, while other

diseases may result in endocarditis, an inflammation of the endocardium or lining membrane of the atrioventricular orifices and operation of the heart. Defects such as the narrowing of the valve stenosis or the defective closing of the valve result in an accumulation of blood in the heart cavity or regurgitation of blood past the valve. If uncorrected, prolonged valvular stenosis or insufficiency may result in cardiac hypertrophy and associated damage to the heart muscle, which may eventually necessitate valve replacement.

The treatment of all these, and other cardiovascular, endothelial and angiogenic disorders, which may or may not be accompanied by cardiac hypertrophy, is encompassed by the present invention.

The terms "cancer", "cancerous", and "malignant" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma including adenocarcinoma, lymphoma, blastoma, melanoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, Hodgkin's and non-Hodgkin's lymphoma, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer such as hepatic carcinoma and hepatoma, bladder cancer, breast cancer, colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer such as renal cell carcinoma and Wilms' tumors, basal cell carcinoma, melanoma, prostate cancer, vulval cancer, thyroid cancer, testicular cancer, esophageal cancer, and various types of head and neck cancer. The preferred cancers for treatment herein are breast, colon, lung, melanoma, ovarian, and others involving vascular tumors as noted above.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., ^{131}I , ^{125}I , ^{90}Y , and ^{186}Re), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents, folic acid antagonists, anti-metabolites of nucleic acid metabolism, antibiotics, pyrimidine analogs, 5-fluorouracil, cisplatin, purine nucleosides, amines, amino acids, triazol nucleosides, or corticosteroids. Specific examples include Adriamycin, Doxorubicin, 5-Fluorouracil, Cytosine arabinoside ("Ara-C"), Cyclophosphamide, Thiotepa, Busulfan, Cytosine, Taxol, Toxotere, Methotrexate, Cisplatin, Melphalan, Vinblastine, Bleomycin, Etoposide, Ifosfamide, Mitomycin C, Mitoxantrone, Vincristine, Vinorelbine, Carboplatin, Teniposide, Daunomycin, Carminomycin, Aminopterin, Dactinomycin, Mitomycins, Esperamicins (see U.S. Pat. No. 4,675,187), Melphalan, and other related nitrogen mustards. Also included in this definition are hormonal agents that act to regulate or inhibit hormone action on tumors, such as tamoxifen and onapristone.

A "growth-inhibitory agent" when used herein refers to a compound or composition that inhibits growth of a cell, such as an Wnt-overexpressing cancer cell, either *in vitro* or *in vivo*. Thus, the growth-inhibitory agent is one which significantly reduces the percentage of malignant cells in S phase. Examples of growth-inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxol, and topo II inhibitors such as doxorubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine,

mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in The Molecular Basis of Cancer, Mendelsohn and Israel, eds., Chapter I, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13. Additional examples include tumor necrosis factor (TNF), an antibody capable of inhibiting or neutralizing the angiogenic activity of acidic or basic FGF or hepatocyte growth factor (HGF), an antibody capable of inhibiting or neutralizing the coagulant activities of tissue factor, protein C, or protein S (*see*, WO 91/01753, published 21 February 1991), or an antibody capable of binding to HER2 receptor (WO 89/06692), such as the 4D5 antibody (and functional equivalents thereof) (*e.g.*, WO 92/22653).

"Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a cardiovascular, endothelial, and angiogenic disorder. The concept of treatment is used in the broadest sense, and specifically includes the prevention (prophylaxis), moderation, reduction, and curing of cardiovascular, endothelial, and angiogenic disorders of any stage. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) a cardiovascular, endothelial, and angiogenic disorder such as hypertrophy. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. The disorder may result from any cause, including idiopathic, cardiotrophic, or myotrophic causes, or ischemia or ischemic insults, such as myocardial infarction.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial effect, such as an anti-hypertrophic effect, for an extended period of time.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, sheep, pigs, etc. Preferably, the mammal is human.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

The phrase "cardiovascular, endothelial or angiogenic agents" refers generically to any drug that acts in treating cardiovascular, endothelial, and angiogenic disorders. Examples of cardiovascular agents are those that promote vascular homeostasis by modulating blood pressure, heart rate, heart contractility, and endothelial and smooth muscle biology, all of which factors have a role in cardiovascular disease. Specific examples of these include angiotensin-II receptor antagonists; endothelin receptor antagonists such as, for example, BOSENTAN™ and MOXONODIN™; interferon-gamma (IFN- γ); des-aspartate-angiotensin I; thrombolytic agents, *e.g.*, streptokinase, urokinase, t-PA, and a t-PA variant specifically designed to have longer half-life and very high fibrin specificity, TNK t-PA (a T103N, N117Q, KHRR(296-299)AAAA t-PA variant, Keyt *et al.*, Proc. Natl. Acad. Sci. USA, 91: 3670-3674 (1994)); inotropic or hypertensive agents such as digoxigenin and β -adrenergic receptor blocking agents, *e.g.*, propranolol, timolol, tertalolol, carteolol, nadolol, betaxolol, penbutolol, acetobutolol, atenolol, metoprolol, and carvedilol; angiotensin converting enzyme (ACE) inhibitors, *e.g.*, quinapril, captopril, enalapril, ramipril, benazepril, fosinopril, and lisinopril; diuretics, *e.g.*, chlorothiazide, hydrochlorothiazide, hydroflumethazide, methylchlorothiazide, benzthiazide, dichlorphenamide, acetazolamide, and indapamide; and calcium channel blockers, *e.g.*, diltiazem, nifedipine, verapamil, nicardipine. One preferred category of this type

is a therapeutic agent used for the treatment of cardiac hypertrophy or of a physiological condition instrumental in the development of cardiac hypertrophy, such as elevated blood pressure, aortic stenosis, or myocardial infarction.

"Angiogenic agents" and "endothelial agents" are active agents that promote angiogenesis and/or endothelial cell growth, or, if applicable, vasculogenesis. This would include factors that accelerate wound healing, such as growth hormone, insulin-like growth factor-I (IGF-I), VEGF, VIGF, PDGF, epidermal growth factor (EGF), CTGF and members of its family, FGF, and TGF- α and TGF- β .

"Angiostatic agents" are active agents that inhibit angiogenesis or vasculogenesis or otherwise inhibit or prevent growth of cancer cells. Examples include antibodies or other antagonists to angiogenic agents as defined above, such as antibodies to VEGF. They additionally include cytotherapeutic agents such as cytotoxic agents, chemotherapeutic agents, growth-inhibitory agents, apoptotic agents, and other agents to treat cancer, such as anti-HER-2, anti-CD20, and other bioactive and organic chemical agents.

In a pharmacological sense, in the context of the present invention, a "therapeutically effective amount" of an active agent such as a PRO polypeptide or agonist or antagonist thereto or an anti-PRO antibody, refers to an amount effective in the treatment of a cardiovascular, endothelial or angiogenic disorder in a mammal and can be determined empirically.

As used herein, an "effective amount" of an active agent such as a PRO polypeptide or agonist or antagonist thereto or an anti-PRO antibody, refers to an amount effective for carrying out a stated purpose, wherein such amounts may be determined empirically for the desired effect.

The terms "PRO polypeptide" and "PRO" as used herein and when immediately followed by a numerical designation refer to various polypeptides, wherein the complete designation (*i.e.*, PRO/number) refers to specific polypeptide sequences as described herein. The terms "PRO/number polypeptide" and "PRO/number" wherein the term "number" is provided as an actual numerical designation as used herein encompass native sequence polypeptides and polypeptide variants (which are further defined herein). The PRO polypeptides described herein may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods.

A "native sequence PRO polypeptide" comprises a polypeptide having the same amino acid sequence as the corresponding PRO polypeptide derived from nature. Such native sequence PRO polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence PRO polypeptide" specifically encompasses naturally-occurring truncated or secreted forms of the specific PRO polypeptide (*e.g.*, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide. In various embodiments of the invention, the native sequence PRO polypeptides disclosed herein are mature or full-length native sequence polypeptides comprising the full-length amino acids sequences shown in the accompanying figures. Start and stop codons are shown in bold font and underlined in the figures. However, while the PRO polypeptide disclosed in the accompanying figures are shown to begin with methionine residues designated herein as amino acid position 1 in the figures, it is conceivable and possible that other methionine residues located either upstream or downstream from the amino acid position 1 in the figures may be employed as the starting amino acid residue for the PRO polypeptides.

The PRO polypeptide "extracellular domain" or "ECD" refers to a form of the PRO polypeptide which is

essentially free of the transmembrane and cytoplasmic domains. Ordinarily, a PRO polypeptide ECD will have less than 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than 0.5% of such domains. It will be understood that any transmembrane domains identified for the PRO polypeptides of the present invention are identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain as initially identified herein. Optionally, therefore, an extracellular domain of a PRO polypeptide may contain from about 5 or fewer amino acids on either side of the transmembrane domain/extracellular domain boundary as identified in the Examples or specification and such polypeptides, with or without the associated signal peptide, and nucleic acid encoding them, are contemplated by the present invention.

The approximate location of the "signal peptides" of the various PRO polypeptides disclosed herein are shown in the present specification and/or the accompanying figures. It is noted, however, that the C-terminal boundary of a signal peptide may vary, but most likely by no more than about 5 amino acids on either side of the signal peptide C-terminal boundary as initially identified herein, wherein the C-terminal boundary of the signal peptide may be identified pursuant to criteria routinely employed in the art for identifying that type of amino acid sequence element (*e.g.*, Nielsen *et al.*, Prot. Eng., 10:1-6 (1997) and von Heinje *et al.*, Nucl. Acids Res., 14:4683-4690 (1986)). Moreover, it is also recognized that, in some cases, cleavage of a signal sequence from a secreted polypeptide is not entirely uniform, resulting in more than one secreted species. These mature polypeptides, where the signal peptide is cleaved within no more than about 5 amino acids on either side of the C-terminal boundary of the signal peptide as identified herein, and the polynucleotides encoding them, are contemplated by the present invention.

"PRO polypeptide variant" means an active PRO polypeptide as defined above or below having at least about 80% amino acid sequence identity with a full-length native sequence PRO polypeptide sequence as disclosed herein, a PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Such PRO polypeptide variants include, for instance, PRO polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the full-length native amino acid sequence. Ordinarily, a PRO polypeptide variant will have at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to a full-length native sequence PRO polypeptide

sequence as disclosed herein, a PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, PRO variant polypeptides are at least about 10 amino acids in length, alternatively at least about 20 amino acids in length, alternatively at least about 30 amino acids in length, alternatively at least about 40 amino acids in length, alternatively at least about 50 amino acids in length, alternatively at least about 60 amino acids in length, alternatively at least about 70 amino acids in length, alternatively at least about 80 amino acids in length, alternatively at least about 90 amino acids in length, alternatively at least about 100 amino acids in length, alternatively at least about 150 amino acids in length, alternatively at least about 200 amino acids in length, alternatively at least about 300 amino acids in length, or more.

"Percent (%) amino acid sequence identity" with respect to the PRO polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in a PRO sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are obtained as described below by using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code shown in Table 1 has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

For purposes herein, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B

to A. As examples of % amino acid sequence identity calculations, Tables 2A-2B demonstrate how to calculate the % amino acid sequence identity of the amino acid sequence designated "Comparison Protein" to the amino acid sequence designated "PRO".

Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described above using the ALIGN-2 sequence comparison computer program. However, % amino acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul *et al.*, Nucleic Acids Res., 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

In addition, % amino acid sequence identity may also be determined using the WU-BLAST-2 computer program (Altschul *et al.*, Methods in Enzymology, 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, *i.e.*, the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. For purposes herein, a % amino acid sequence identity value is determined by dividing (a) the number of matching identical amino acids residues between the amino acid sequence of the PRO polypeptide of interest having a sequence derived from the native PRO polypeptide and the comparison amino acid sequence of interest (*i.e.*, the sequence against which the PRO polypeptide of interest is being compared which may be a PRO variant polypeptide) as determined by WU-BLAST-2 by (b) the total number of amino acid residues of the PRO polypeptide of interest. For example, in the statement "a polypeptide comprising an amino acid sequence A which has or having at least 80% amino acid sequence identity to the amino acid sequence B", the amino acid sequence A is the comparison amino acid sequence of interest and the amino acid sequence B is the amino acid sequence of the PRO polypeptide of interest.

"PRO variant polynucleotide" or "PRO variant nucleic acid sequence" means a nucleic acid molecule which

encodes an active PRO polypeptide as defined below and which has at least about 80% nucleic acid sequence identity with a nucleotide acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, a PRO variant polynucleotide will have at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity with a nucleic acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal sequence, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Variants do not encompass the native nucleotide sequence.

Ordinarily, PRO variant polynucleotides are at least about 30 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 120 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 210 nucleotides in length, alternatively at least about 240 nucleotides in length, alternatively at least about 270 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 900 nucleotides in length, or more.

"Percent (%) nucleic acid sequence identity" with respect to the PRO polypeptide-encoding nucleic acid sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in a PRO polypeptide-encoding nucleic acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For purposes herein, however, % nucleic acid sequence identity values are obtained as described below by using the sequence comparison computer program ALIGN-2, wherein the complete source code for the

ALIGN-2 program is provided in Table 1. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code shown in Table 1 has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

For purposes herein, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

$$100 \text{ times the fraction } W/Z$$

where W is the number of nucleotides scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C. As examples of % nucleic acid sequence identity calculations, Tables 2C-2D demonstrate how to calculate the % nucleic acid sequence identity of the nucleic acid sequence designated "Comparison DNA" to the nucleic acid sequence designated "PRO-DNA".

Unless specifically stated otherwise, all % nucleic acid sequence identity values used herein are obtained as described above using the ALIGN-2 sequence comparison computer program. However, % nucleic acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul *et al.*, Nucleic Acids Res., 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

$$100 \text{ times the fraction } W/Z,$$

where W is the number of nucleotides scored as identical matches by the sequence alignment program NCBI-

BLAST2 in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C.

In addition, % nucleic acid sequence identity values may also be generated using the WU-BLAST-2 computer program (Altschul *et al.*, Methods in Enzymology, 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, *i.e.*, the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. For purposes herein, a % nucleic acid sequence identity value is determined by dividing (a) the number of matching identical nucleotides between the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest having a sequence derived from the native sequence PRO polypeptide-encoding nucleic acid and the comparison nucleic acid molecule of interest (*i.e.*, the sequence against which the PRO polypeptide-encoding nucleic acid molecule of interest is being compared which may be a variant PRO polynucleotide) as determined by WU-BLAST-2 by (b) the total number of nucleotides of the PRO polypeptide-encoding nucleic acid molecule of interest. For example, in the statement "an isolated nucleic acid molecule comprising a nucleic acid sequence A which has or having at least 80% nucleic acid sequence identity to the nucleic acid sequence B", the nucleic acid sequence A is the comparison nucleic acid molecule of interest and the nucleic acid sequence B is the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest.

In other embodiments, PRO variant polynucleotides are nucleic acid molecules that encode an active PRO polypeptide and which are capable of hybridizing, preferably under stringent hybridization and wash conditions, to nucleotide sequences encoding the full-length PRO polypeptide as shown in the specification herein and accompanying figures. PRO variant polypeptides may be those that are encoded by a PRO variant polynucleotide.

The term "positives", in the context of the amino acid sequence identity comparisons performed as described above, includes amino acid residues in the sequences compared that are not only identical, but also those that have similar properties. Amino acid residues that score a positive value to an amino acid residue of interest are those that are either identical to the amino acid residue of interest or are a preferred substitution (as defined in Table 3 below) of the amino acid residue of interest.

For purposes herein, the % value of positives of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % positives to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scoring a positive value by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % positives of A to B will not equal the % positives of B to A.

"Isolated", when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Preferably, the isolated

polypeptide is free of association with all components with which it is naturally associated. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the PRO natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" nucleic acid molecule encoding a PRO polypeptide or an "isolated" nucleic acid molecule encoding an anti-PRO antibody is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the PRO-encoding nucleic acid or the natural source of the anti-PRO-encoding nucleic acid. Preferably, the isolated nucleic acid is free of association with all components with which it is naturally associated. An isolated PRO-encoding nucleic acid molecule or an isolated anti-PRO-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the PRO-encoding nucleic acid molecule or from the anti-PRO-encoding nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule encoding a PRO polypeptide or an isolated nucleic acid molecule encoding an anti-PRO antibody includes PRO-nucleic acid molecules or anti-PRO-nucleic acid molecules contained in cells that ordinarily express PRO polypeptides or anti-PRO antibodies where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a PRO polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are

present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature that can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, *see*, Ausubel *et al.*, Current Protocols in Molecular Biology (Wiley Interscience Publishers, 1995).

"Stringent conditions" or "high-stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example, 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately-stringent conditions" may be identified as described by Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Press, 1989), and include the use of washing solution and hybridization conditions (*e.g.*, temperature, ionic strength, and % SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

The modifier "epitope-tagged" when used herein refers to a chimeric polypeptide comprising a PRO polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

"Active" or "activity" in the context of PRO variants refers to form(s) of PRO proteins that retain the biologic and/or immunologic activities of a native or naturally-occurring PRO polypeptide.

"Biological activity" in the context of a molecule that antagonizes a PRO polypeptide that can be identified by the screening assays disclosed herein (*e.g.*, an organic or inorganic small molecule, peptide, etc.) is used to refer to the ability of such molecules to bind or complex with the PRO polypeptide identified herein, or otherwise interfere with the interaction of the PRO polypeptides with other cellular proteins or otherwise inhibits the transcription or translation of the PRO polypeptide. Particularly preferred biological activity includes cardiac hypertrophy, activity that acts on systemic disorders that affect vessels, such as diabetes mellitus, as well as diseases of the arteries, capillaries, veins, and/or lymphatics, and cancer.

The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes one or more of the biological activities of a native PRO polypeptide disclosed herein, for example, if applicable, its mitogenic or angiogenic activity. Antagonists of a PRO polypeptide may act by interfering with the binding of a PRO polypeptide to a cellular receptor, by incapacitating or killing cells that have been activated by a PRO polypeptide, or by interfering with vascular endothelial cell activation after binding of a PRO polypeptide to a cellular receptor. All such points of intervention by a PRO polypeptide antagonist shall be considered equivalent for purposes of this invention. The antagonists inhibit the mitogenic, angiogenic, or other biological activity of PRO polypeptides, and thus are useful for the treatment of diseases or disorders characterized by undesirable excessive neovascularization, including by way of example tumors, and especially solid malignant tumors, rheumatoid arthritis, psoriasis, atherosclerosis, diabetic and other retinopathies, retrolental fibroplasia, age-related macular degeneration, neovascular glaucoma, hemangiomas, thyroid hyperplasias (including Grave's disease), corneal and other tissue transplantation, and chronic inflammation. The antagonists also are useful for the treatment of diseases or disorders characterized by undesirable excessive vascular permeability, such as edema associated with brain tumors, ascites associated with malignancies, Meigs' syndrome, lung inflammation, nephrotic syndrome, pericardial effusion (such as that associated with pericarditis), and pleural effusion. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native PRO polypeptide disclosed herein. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments, or amino acid sequence variants of native PRO polypeptides, peptides, small organic molecules, etc.

A "small molecule" is defined herein to have a molecular weight below about 500 daltons.

The term "PRO polypeptide receptor" as used herein refers to a cellular receptor for a PRO polypeptide, ordinarily a cell-surface receptor found on vascular endothelial cells, as well as variants thereof that retain the ability to bind a PRO polypeptide.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules that lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas. The term "antibody" is used in the broadest sense and specifically covers, without limitation, intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies) formed from at least two intact antibodies, and antibody fragments, so long as they exhibit the desired biological activity.

"Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form

an interface between the light- and heavy-chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody to and for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies.

5 It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β -sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β -sheet structure. The CDRs in each chain are held together in
10 close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies. See, Kabat *et al.*, NIH Publ. No.91-3242, Vol. I, pages 647-669 (1991). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen-binding or variable
15 region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies (Zapata *et al.*, Protein Eng., 8(10): 1057-1062 (1995)); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily.
20 Pepsin treatment yields an F(ab')₂ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment that contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody.
25 However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.
30

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM; and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2.

The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

5 The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, 10 the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, Nature, 256: 495 (1975), 15 or may be made by recombinant DNA methods (*see, e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, Nature, 352: 624-628 (1991) and Marks *et al.*, J. Mol. Biol., 222: 581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies 20 derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity. U.S. Patent No. 4,816,567; Morrison *et al.*, Proc. Natl. Acad. Sci. USA, 81: 6851-6855 (1984).

25 "Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired 30 specificity, affinity, and capacity. In some instances, Fv FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR 35 regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody preferably also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, *see* Jones *et al.*, Nature, 321: 522-525 (1986); Reichmann *et al.*, Nature, 332: 323-329 (1988); and Presta, Curr. Op.

Struct. Biol., 2: 593-596 (1992). The humanized antibody includes a PRIMATIZED™ antibody wherein the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest.

5 "Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains that enables the sFv to form the desired structure for antigen binding. For a review of sFv see, Pluckthun in The Pharmacology of Monoclonal Antibodies, Vol. 113, Rosenberg and Moore, eds. (Springer-Verlag: New York, 1994), pp. 269-315.

10 The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, Proc. Natl. Acad. Sci. USA, 90: 6444-6448 (1993).

15 An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells, since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

25 The word "label" when used herein refers to a detectable compound or other composition that is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition that is detectable. Radionuclides that can serve as detectable labels include, for example, I-131, I-123, I-125, Y-90, Re-188, At-211, Cu-67, Bi-212, and Pd-109. The label may also be a non-detectable entity such as a toxin.

30 By "solid phase" is meant a non-aqueous matrix to which an antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g., an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

35 A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant that is useful for delivery of a drug (such as the PRO polypeptide or antibodies thereto disclosed herein) to a mammal.

The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

As used herein, the term "immunoadhesin" designates antibody-like molecules that combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity that is other than the antigen recognition and binding site of an antibody (*i.e.*, is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD, or IgM.

As shown below, Table 1 provides the complete source code for the ALIGN-2 sequence comparison computer program. This source code may be routinely compiled for use on a UNIX operating system to provide the ALIGN-2 sequence comparison computer program.

In addition, Tables 2A-2D show hypothetical exemplifications for using the below described method to determine % amino acid sequence identity (Tables 2A-2B) and % nucleic acid sequence identity (Tables 2C-2D) using the ALIGN-2 sequence comparison computer program, wherein "PRO" represents the amino acid sequence of a hypothetical PRO polypeptide of interest, "Comparison Protein" represents the amino acid sequence of a polypeptide against which the "PRO" polypeptide of interest is being compared, "PRO-DNA" represents a hypothetical PRO-encoding nucleic acid sequence of interest, "Comparison DNA" represents the nucleotide sequence of a nucleic acid molecule against which the "PRO-DNA" nucleic acid molecule of interest is being compared, "X", "Y", and "Z" each represent different hypothetical amino acid residues and "N", "L" and "V" each represent different hypothetical nucleotides.

Table 1

```

/*
 *
 * C-C increased from 12 to 15
 * Z is average of EQ
 / * B is average of ND
 * match with stop is _M; stop-stop = 0; J (joker) match = 0
 */
#define _M -8 /* value of a match with a stop */

int _day[26][26] = {
/* A B C D E F G H I J K L M N O P Q R S T U V W X Y Z */
/* A */ { 2, 0, -2, 0, 0, -4, 1, -1, -1, 0, -1, -2, -1, 0, _M, 1, 0, -2, 1, 1, 0, 0, -6, 0, -3, 0},
/* B */ { 0, 3, -4, 3, 2, -5, 0, 1, -2, 0, 0, -3, -2, 2, _M, -1, 1, 0, 0, 0, -2, -5, 0, -3, 1},
/* C */ { -2, -4, 15, -5, -5, -4, -3, -3, -2, 0, -5, -6, -5, -4, _M, -3, -5, -4, 0, -2, 0, -2, -8, 0, 0, -5},
/* D */ { 0, 3, -5, 4, 3, -6, 1, 1, -2, 0, 0, -4, -3, 2, _M, -1, 2, -1, 0, 0, 0, -2, -7, 0, -4, 2},
/* E */ { 0, 2, -5, 3, 4, -5, 0, 1, -2, 0, 0, -3, -2, 1, _M, -1, 2, -1, 0, 0, 0, -2, -7, 0, -4, 3},
/* F */ { -4, -5, -4, -6, -5, 9, -5, -2, 1, 0, -5, 2, 0, -4, _M, -5, -5, -4, -3, -3, 0, -1, 0, 0, 7, -5},
/* G */ { 1, 0, -3, 1, 0, -5, 5, -2, -3, 0, -2, -4, -3, 0, _M, -1, -1, -3, 1, 0, 0, -1, -7, 0, -5, 0},
/* H */ { -1, 1, -3, 1, 1, -2, -2, 6, -2, 0, 0, -2, -2, 2, _M, 0, 3, 2, -1, -1, 0, -2, -3, 0, 0, 2},
/* I */ { -1, -2, -2, -2, -2, 1, -3, -2, 5, 0, -2, 2, 2, -2, _M, -2, -2, -2, -1, 0, 0, 4, -5, 0, -1, -2},
/* J */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* K */ { -1, 0, -5, 0, 0, -5, -2, 0, -2, 0, 5, -3, 0, 1, _M, -1, 1, 3, 0, 0, 0, -2, -3, 0, -4, 0},
/* L */ { -2, -3, -6, -4, -3, 2, -4, -2, 2, 0, -3, 6, 4, -3, _M, -3, -2, -3, -3, -1, 0, 2, -2, 0, -1, -2},
/* M */ { -1, -2, -5, -3, -2, 0, -3, -2, 2, 0, 0, 4, 6, -2, _M, -2, -1, 0, -2, -1, 0, 2, -4, 0, -2, -1},
/* N */ { 0, 2, -4, 2, 1, -4, 0, 2, -2, 0, 1, -3, -2, 2, _M, -1, 1, 0, 1, 0, 0, -2, -4, 0, -2, 1},
/* O */ { _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M},
/* P */ { 1, -1, -3, -1, -1, -5, -1, 0, -2, 0, -1, -3, -2, -1, _M, 6, 0, 0, 1, 0, 0, -1, -6, 0, -5, 0},
/* Q */ { 0, 1, -5, 2, 2, -5, -1, 3, -2, 0, 1, -2, -1, 1, _M, 0, 4, 1, -1, -1, 0, -2, -5, 0, -4, 3},
/* R */ { -2, 0, -4, -1, -1, -4, -3, 2, -2, 0, 3, -3, 0, 0, _M, 0, 1, 6, 0, -1, 0, -2, 2, 0, -4, 0},
/* S */ { 1, 0, 0, 0, 0, -3, 1, -1, -1, 0, 0, -3, -2, 1, _M, 1, -1, 0, 2, 1, 0, -1, -2, 0, -3, 0},
/* T */ { 1, 0, -2, 0, 0, -3, 0, -1, 0, 0, 0, -1, -1, 0, _M, 0, -1, -1, 1, 3, 0, 0, -5, 0, -3, 0},
/* U */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* V */ { 0, -2, -2, -2, -2, -1, -1, -2, 4, 0, -2, 2, 2, -2, _M, -1, -2, -2, -1, 0, 0, 4, -6, 0, -2, -2},
/* W */ { -6, -5, -8, -7, -7, 0, -7, -3, -5, 0, -3, -2, -4, -4, _M, -6, -5, 2, -2, -5, 0, -6, 17, 0, 0, -6},
/* X */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* Y */ { -3, -3, 0, -4, -4, 7, -5, 0, -1, 0, -4, -1, -2, -2, _M, -5, -4, -4, -3, -3, 0, -2, 0, 0, 10, -4},
/* Z */ { 0, 1, -5, 2, 3, -5, 0, 2, -2, 0, 0, -2, -1, 1, _M, 0, 3, 0, 0, 0, 0, -2, -6, 0, -4, 4}
};

```

Table 1 (cont')

```

/*
*/
#include <stdio.h>
#include <ctype.h>

#define MAXJMP      16      /* max jumps in a diag */
#define MAXGAP      24      /* don't continue to penalize gaps larger than this */
#define JMPS        1024    /* max jmps in an path */
#define MX          4       /* save if there's at least MX-1 bases since last jmp */

#define DMAT        3       /* value of matching bases */
#define DMIS        0       /* penalty for mismatched bases */
#define DINS0       8       /* penalty for a gap */
#define DINS1       1       /* penalty per base */
#define PINS0       8       /* penalty for a gap */
#define PINS1       4       /* penalty per residue */

struct jmp {
    short          n[MAXJMP];    /* size of jmp (neg for dely) */
    unsigned short x[MAXJMP];    /* base no. of jmp in seq x */
};
/* limits seq to 2^16 -1 */

struct diag {
    int            score;        /* score at last jmp */
    long           offset;       /* offset of prev block */
    short          ijmp;         /* current jmp index */
    struct jmp     jp;           /* list of jmps */
};

struct path {
    int            spc;          /* number of leading spaces */
    short          n[JMPS];      /* size of jmp (gap) */
    int            x[JMPS];      /* loc of jmp (last elem before gap) */
};

char *ofile;                  /* output file name */
char *namex[2];               /* seq names: getseqs() */
char *prog;                   /* prog name for err msgs */
char *seqx[2];                 /* seqs: getseqs() */
int dmax;                      /* best diag: nw() */
int dmax0;                     /* final diag */
int dna;                       /* set if dna: main() */
int endgaps;                   /* set if penalizing end gaps */
int gapx, gapy;                /* total gaps in seqs */
int len0, len1;                /* seq lens */
int ngapx, ngapy;              /* total size of gaps */
int smax;                      /* max score: nw() */
int *xbm;                      /* bitmap for matching */
long offset;                   /* current offset in jmp file */
struct diag *dx;               /* holds diagonals */
struct path pp[2];             /* holds path for seqs */

char *calloc(), *malloc(), *index(), *strcpy();
char *getseq(), *g_calloc();

```

Table 1 (cont')

```

/* Needleman-Wunsch alignment program
*
* usage: progs file1 file2
* where file1 and file2 are two dna or two protein sequences.
* The sequences can be in upper- or lower-case and may contain ambiguity
* Any lines beginning with ';', '>' or '<' are ignored
* Max file length is 65535 (limited by unsigned short x in the jmp struct)
* A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
* Output is in the file "align.out"
*
* The program may create a tmp file in /tmp to hold info about traceback.
* Original version developed under BSD 4.3 on a vax 8650
*/
#include "nw.h"
#include "day.h"

static _dbval[26] = {
    1,14,2,13,0,0,4,11,0,0,12,0,3,15,0,0,0,5,6,8,8,7,9,0,10,0
};

static _pbval[26] = {
    1, 2|(1<<('D'-'A'))|(1<<('N'-'A')), 4, 8, 16, 32, 64,
    128, 256, 0xFFFFFFFF, 1<<10, 1<<11, 1<<12, 1<<13, 1<<14,
    1<<15, 1<<16, 1<<17, 1<<18, 1<<19, 1<<20, 1<<21, 1<<22,
    1<<23, 1<<24, 1<<25|(1<<('E'-'A'))|(1<<('Q'-'A'))
};

main(ac, av)
    int ac;
    char *av[];
{
    prog = av[0];
    if (ac != 3) {
        fprintf(stderr, "usage: %s file1 file2\n", prog);
        fprintf(stderr, "where file1 and file2 are two dna or two protein sequences.\n");
        fprintf(stderr, "The sequences can be in upper- or lower-case\n");
        fprintf(stderr, "Any lines beginning with ';' or '<' are ignored\n");
        fprintf(stderr, "Output is in the file \"align.out\"\n");
        exit(1);
    }
    namex[0] = av[1];
    namex[1] = av[2];
    seqx[0] = getseq(namex[0], &len0);
    seqx[1] = getseq(namex[1], &len1);
    xbm = (dna)? _dbval : _pbval;

    endgaps = 0; /* 1 to penalize endgaps */
    ofile = "align.out"; /* output file */

    nw(); /* fill in the matrix, get the possible jumps */
    readjumps(); /* get the actual jumps */
    print(); /* print stats, alignment */

    cleanup(); /* unlink any tmp files */
}

```

main

Table 1 (cont')

```

/* do the alignment, return best score: main()
 * dna: values in Fitch and Smith, PNAS, 80, 1382-1386, 1983
 * pro: PAM 250 values
 * When scores are equal, we prefer mismatches to any gap, prefer
 * a new gap to extending an ongoing gap, and prefer a gap in seqx
 * to a gap in seq y.
 */
nw()
{
    char      *px, *py;          /* seqs and ptrs */
    int      *ndely, *dely;      /* keep track of dely */
    int      ndelx, delx;        /* keep track of delx */
    int      *tmp;              /* for swapping row0, row1 */
    int      mis;               /* score for each type */
    int      ins0, ins1;        /* insertion penalties */
    register  id;               /* diagonal index */
    register  ij;               /* jmp index */
    register  *col0, *col1;      /* score for curr, last row */
    register  xx, yy;           /* index into seqs */

    dx = (struct diag *)g_calloc("to get diags", len0 + len1 + 1, sizeof(struct diag));

    ndely = (int *)g_calloc("to get ndely", len1 + 1, sizeof(int));
    dely = (int *)g_calloc("to get dely", len1 + 1, sizeof(int));
    col0 = (int *)g_calloc("to get col0", len1 + 1, sizeof(int));
    col1 = (int *)g_calloc("to get col1", len1 + 1, sizeof(int));
    ins0 = (dna)? DINS0 : PINS0;
    ins1 = (dna)? DINS1 : PINS1;

    smax = -10000;
    if (endgaps) {
        for (col0[0] = dely[0] = -ins0, yy = 1; yy <= len1; yy++) {
            col0[yy] = dely[yy] = col0[yy-1] - ins1;
            ndely[yy] = yy;
        }
        col0[0] = 0;          /* Waterman Bull Math Biol 84 */
    }
    else
        for (yy = 1; yy <= len1; yy++)
            dely[yy] = -ins0;

    /* fill in match matrix
    */
    for (px = seqx[0], xx = 1; xx <= len0; px++, xx++) {
        /* initialize first entry in col
        */
        if (endgaps) {
            if (xx == 1)
                col1[0] = delx = -(ins0 + ins1);
            else
                col1[0] = delx = col0[0] - ins1;
            ndelx = xx;
        }
        else {
            col1[0] = 0;
            delx = -ins0;
            ndelx = 0;
        }
    }
}

```

nw

Table 1 (cont')

...nw

```

for (py = seqx[1], yy = 1; yy <= len1; py++, yy++) {
    mis = col0[yy-1];
    if (dna)
        mis += (xbm[*px-'A']&xbm[*py-'A'])? DMAT : DMIS;
    else
        mis += _day[*px-'A'][*py-'A'];

    /* update penalty for del in x seq;
     * favor new del over ongong del
     * ignore MAXGAP if weighting endgaps
     */
    if (endgaps || ndely[yy] < MAXGAP) {
        if (col0[yy] - ins0 >= dely[yy]) {
            dely[yy] = col0[yy] - (ins0+ins1);
            ndely[yy] = 1;
        } else {
            dely[yy] -= ins1;
            ndely[yy]++;
        }
    } else {
        if (col0[yy] - (ins0+ins1) >= dely[yy]) {
            dely[yy] = col0[yy] - (ins0+ins1);
            ndely[yy] = 1;
        } else
            ndely[yy]++;
    }

    /* update penalty for del in y seq;
     * favor new del over ongong del
     */
    if (endgaps || ndelx < MAXGAP) {
        if (col1[yy-1] - ins0 >= delx) {
            delx = col1[yy-1] - (ins0+ins1);
            ndelx = 1;
        } else {
            delx -= ins1;
            ndelx++;
        }
    } else {
        if (col1[yy-1] - (ins0+ins1) >= delx) {
            delx = col1[yy-1] - (ins0+ins1);
            ndelx = 1;
        } else
            ndelx++;
    }

    /* pick the maximum score; we're favoring
     * mis over any del and delx over dely
     */

```

Table 1 (cont')

...nw

```

id = xx - yy + len1 - 1;
if (mis >= delx && mis >= dely[yy])
    coll[yy] = mis;
else if (delx >= dely[yy]) {
    coll[yy] = delx;
    ij = dx[id].ijmp;
    if (dx[id].jp.n[0] && (!dna || (ndelx >= MAXJMP
    && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
        dx[id].ijmp++;
        if (++ij >= MAXJMP) {
            writeimps(id);
            ij = dx[id].ijmp = 0;
            dx[id].offset = offset;
            offset += sizeof(struct jmp) + sizeof(offset);
        }
        dx[id].jp.n[ij] = ndelx;
        dx[id].jp.x[ij] = xx;
        dx[id].score = delx;
    }
    else {
        coll[yy] = dely[yy];
        ij = dx[id].ijmp;
        if (dx[id].jp.n[0] && (!dna || (ndely[yy] >= MAXJMP
        && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
            dx[id].ijmp++;
            if (++ij >= MAXJMP) {
                writeimps(id);
                ij = dx[id].ijmp = 0;
                dx[id].offset = offset;
                offset += sizeof(struct jmp) + sizeof(offset);
            }
            dx[id].jp.n[ij] = -ndely[yy];
            dx[id].jp.x[ij] = xx;
            dx[id].score = dely[yy];
        }
        if (xx == len0 && yy < len1) {
            /* last col
            */
            if (endgaps)
                coll[yy] -= ins0+ins1*(len1-yy);
            if (coll[yy] > smax) {
                smax = coll[yy];
                dmax = id;
            }
        }
    }
}
if (endgaps && xx < len0)
    coll[yy-1] -= ins0+ins1*(len0-xx);
if (coll[yy-1] > smax) {
    smax = coll[yy-1];
    dmax = id;
}
tmp = col0; col0 = coll; coll = tmp;
}
(void) free((char *)ndely);
(void) free((char *)dely);
(void) free((char *)col0);
(void) free((char *)coll);
}

```

Table 1 (cont')

```

/*
 *
 * print() -- only routine visible outside this module
 *
 * static:
 * getmat() -- trace back best path, count matches: print()
 * pr_align() -- print alignment of described in array p[]: print()
 * dumpblock() -- dump a block of lines with numbers, stars: pr_align()
 * nums() -- put out a number line: dumpblock()
 * putline() -- put out a line (name, [num], seq, [num]): dumpblock()
 * stars() -- put a line of stars: dumpblock()
 * stripname() -- strip any path and prefix from a seqname
 */

#include "nw.h"

#define SPC      3
#define P_LINE  256 /* maximum output line */
#define P_SPC    3   /* space between name or num and seq */

extern _day[26][26];
int olen; /* set output line length */
FILE *fx; /* output file */

print()
{
    int lx, ly, firstgap, lastgap; /* overlap */

    if ((fx = fopen(ofile, "w")) == 0) {
        fprintf(stderr, "%s: can't write %s\n", prog, ofile);
        cleanup(1);
    }
    fprintf(fx, "< first sequence: %s (length = %d)\n", namex[0], len0);
    fprintf(fx, "< second sequence: %s (length = %d)\n", namex[1], len1);
    olen = 60;
    lx = len0;
    ly = len1;
    firstgap = lastgap = 0;
    if (dmax < len1 - 1) { /* leading gap in x */
        pp[0].spc = firstgap = len1 - dmax - 1;
        ly -= pp[0].spc;
    }
    else if (dmax > len1 - 1) { /* leading gap in y */
        pp[1].spc = firstgap = dmax - (len1 - 1);
        lx -= pp[1].spc;
    }
    if (dmax0 < len0 - 1) { /* trailing gap in x */
        lastgap = len0 - dmax0 - 1;
        lx -= lastgap;
    }
    else if (dmax0 > len0 - 1) { /* trailing gap in y */
        lastgap = dmax0 - (len0 - 1);
        ly -= lastgap;
    }
    getmat(lx, ly, firstgap, lastgap);
    pr_align();
}

```

print

Table 1 (cont')

```

/*
 * trace back the best path, count matches
 */
static
getmat(lx, ly, firstgap, lastgap)                                getmat
{
    int lx, ly;                                                    /* "core" (minus endgaps) */
    int firstgap, lastgap;                                          /* leading trailing overlap */

    int nm, i0, i1, siz0, siz1;
    char outx[32];
    double pct;
    register n0, n1;
    register char *p0, *p1;

    /* get total matches, score
    */
    i0 = i1 = siz0 = siz1 = 0;
    p0 = seqx[0] + pp[1].spc;
    p1 = seqx[1] + pp[0].spc;
    n0 = pp[1].spc + 1;
    n1 = pp[0].spc + 1;

    nm = 0;
    while ( *p0 && *p1 ) {
        if (siz0) {
            p1++;
            n1++;
            siz0--;
        }
        else if (siz1) {
            p0++;
            n0++;
            siz1--;
        }
        else {
            if (xbm[*p0-'A'] & xbm[*p1-'A'])
                nm++;
            if (n0++ == pp[0].x[i0])
                siz0 = pp[0].n[i0++];
            if (n1++ == pp[1].x[i1])
                siz1 = pp[1].n[i1++];
            p0++;
            p1++;
        }
    }

    /* pct homology:
    * if penalizing endgaps, base is the shorter seq
    * else, knock off overhangs and take shorter core
    */
    if (endgaps)
        lx = (len0 < len1)? len0 : len1;
    else
        lx = (lx < ly)? lx : ly;
    pct = 100.*((double)nm)/((double)lx);
    fprintf(fx, "\n");
    fprintf(fx, "< %d match%s in an overlap of %d: %.2f percent similarity\n",
        nm, (nm == 1)? "" : "es", lx, pct);

```

Table 1 (cont')

```

fprintf(fx, "< gaps in first sequence: %d", gapx);
if (gapx) {
    (void) sprintf(outx, " (%d %s%s)",
        ngapx, (dna)? "base": "residue", (ngapx == 1)? "": "s");
    fprintf(fx, "%s", outx);

    fprintf(fx, ", gaps in second sequence: %d", gapy);
    if (gapy) {
        (void) sprintf(outx, " (%d %s%s)",
            ngapy, (dna)? "base": "residue", (ngapy == 1)? "": "s");
        fprintf(fx, "%s", outx);
    }
    if (dna)
        fprintf(fx,
            "\n< score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per base)\n",
            smax, DMAT, DMIS, DINS0, DINS1);
    else
        fprintf(fx,
            "\n< score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per residue)\n",
            smax, PINS0, PINS1);
    if (endgaps)
        fprintf(fx,
            "< endgaps penalized. left endgap: %d %s%s, right endgap: %d %s%s\n",
            firstgap, (dna)? "base": "residue", (firstgap == 1)? "": "s",
            lastgap, (dna)? "base": "residue", (lastgap == 1)? "": "s");
    else
        fprintf(fx, "< endgaps not penalized\n");
}

static      nm;          /* matches in core -- for checking */
static      lmax;        /* lengths of stripped file names */
static      ij[2];       /* jmp index for a path */
static      nc[2];       /* number at start of current line */
static      ni[2];       /* current elem number -- for gapping */
static      siz[2];
static char *ps[2];      /* ptr to current element */
static char *po[2];      /* ptr to next output char slot */
static char out[2][P_LINE]; /* output line */
static char star[P_LINE]; /* set by stars() */

/*
 * print alignment of described in struct path pp[]
 */
static
pr_align()
{
    int      nn;          /* char count */
    int      more;
    register i;

    for (i = 0, lmax = 0; i < 2; i++) {
        nn = stripname(name[i]);
        if (nn > lmax)
            lmax = nn;

        nc[i] = 1;
        ni[i] = 1;
        siz[i] = ij[i] = 0;
        ps[i] = seqx[i];
        po[i] = out[i];
    }
}

```

...getmat

pr_align

Table 1 (cont')

```

for (nn = nm = 0, more = 1; more; ) {
    for (i = more = 0; i < 2; i++) {
        /*
         * do we have more of this sequence?
         */
        if (!*ps[i])
            continue;

        more++;

        if (pp[i].spc) { /* leading space */
            *po[i]++ = ' ';
            pp[i].spc--;
        }
        else if (siz[i]) { /* in a gap */
            *po[i]++ = '-';
            siz[i]--;
        }
        else { /* we're putting a seq element
            */
            *po[i] = *ps[i];
            if (islower(*ps[i]))
                *ps[i] = toupper(*ps[i]);
            po[i]++;
            ps[i]++;

            /*
             * are we at next gap for this seq?
             */
            if (ni[i] == pp[i].x[ij[i]]) {
                /*
                 * we need to merge all gaps
                 * at this location
                 */
                siz[i] = pp[i].n[ij[i] + +];
                while (ni[i] == pp[i].x[ij[i]])
                    siz[i] += pp[i].n[ij[i] + +];
            }
            ni[i]++;
        }
    }
    if (++nn == olen || !more && nn) {
        dumpblock();
        for (i = 0; i < 2; i++)
            po[i] = out[i];
        nn = 0;
    }
}

/*
 * dump a block of lines, including numbers, stars: pr_align()
 */
static
dumpblock()
{
    register i;

    for (i = 0; i < 2; i++)
        *po[i] = '\0';
}

```

...pr_align

dumpblock

Table 1 (cont')

...dumpblock

```

(void) putc('\n', fx);
for (i = 0; i < 2; i++) {
    if (*out[i] && (*out[i] != ' ' || *(po[i]) != ' ')) {
        if (i == 0)
            nums(i);
        if (i == 0 && *out[1])
            stars();
        putline(i);
        if (i == 0 && *out[1])
            fprintf(fx, star);
        if (i == 1)
            nums(i);
    }
}

/*
 * put out a number line: dumpblock()
 */
static
nums(ix)
int ix;    /* index in out[] holding seq line */
{
    char        nline[P_LINE];
    register    i, j;
    register char *pn, *px, *py;

    for (pn = nline, i = 0; i < lmax + P_SPC; i++, pn++)
        *pn = ' ';
    for (i = nc[ix], py = out[ix]; *py; py++, pn++) {
        if (*py == ' ' || *py == '-')
            *pn = ' ';
        else {
            if (i%10 == 0 || (i == 1 && nc[ix] != 1)) {
                j = (i < 0)? -i : i;
                for (px = pn; j; j /= 10, px--)
                    *px = j%10 + '0';
                if (i < 0)
                    *px = '-';
            }
            else
                *pn = ' ';
            i++;
        }
    }
    *pn = '\0';
    nc[ix] = i;
    for (pn = nline; *pn; pn++)
        (void) putc(*pn, fx);
    (void) putc('\n', fx);
}

/*
 * put out a line (name, {num}, seq, {num}): dumpblock()
 */
static
putline(ix)
int ix;

```

nums

putline

Table 1 (cont')

...putline

```

int      i;
register char      *px;

for (px = namex[ix], i = 0; *px && *px != ':'; px++, i++)
    (void) putc(*px, fx);
for (; i < lmax+P_SPC; i++)
    (void) putc(' ', fx);

/* these count from 1:
 * ni[] is current element (from 1)
 * nc[] is number at start of current line
 */
for (px = out[ix]; *px; px++)
    (void) putc(*px&0x7F, fx);
(void) putc('\n', fx);
}

```

```

/*
 * put a line of stars (seqs always in out[0], out[1]): dumpblock()
 */
static
stars()
{
    int      i;
    register char      *p0, *p1, cx, *px;

    if (!*out[0] || (*out[0] == ' ' && *(po[0]) == ' ') ||
        !*out[1] || (*out[1] == ' ' && *(po[1]) == ' '))
        return;
    px = star;
    for (i = lmax+P_SPC; i; i--)
        *px++ = ' ';

    for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
        if (isalpha(*p0) && isalpha(*p1)) {
            if (xbm[*p0-'A']&xbm[*p1-'A']) {
                cx = '*';
                nm++;
            }
            else if (!dna && _day[*p0-'A'][*p1-'A'] > 0)
                cx = '.';
            else
                cx = ' ';
        }
        else
            cx = ' ';
        *px++ = cx;
    }
    *px++ = '\n';
    *px = '\0';
}

```

stars

Table 1 (cont')

```
/*  
 * strip path or prefix from pn, return len: pr_align()  
 */  
static  
stripname(pn)  
{  
    char      *pn;    /* file name (may be path) */  
  
    register char      *px, *py;  
  
    py = 0;  
    for (px = pn; *px; px++)  
        if (*px == '/')  
            py = px + 1;  
    if (py)  
        (void) strcpy(pn, py);  
    return(strlen(pn));  
}
```

stripname

Table 1 (cont')

```

/*
 * cleanup() -- cleanup any tmp file
 * getseq() -- read in seq, set dna, len, maxlen
 * g_calloc() -- calloc() with error checkin
 * readjumps() -- get the good jumps, from tmp file if necessary
 * writejumps() -- write a filled array of jumps to a tmp file: nw()
 */
#include "nw.h"
#include <sys/file.h>

char *jname = "/tmp/homgXXXXXX"; /* tmp file for jumps */
FILE *fj;

int cleanup(); /* cleanup tmp file */
long lseek();

/*
 * remove any tmp file if we blow
 */
cleanup(i)
    int i;
{
    if (fj)
        (void) unlink(jname);
    exit(i);
}

/*
 * read, return ptr to seq, set dna, len, maxlen
 * skip lines starting with ';', '<', or '>'
 * seq in upper or lower case
 */
char *
getseq(file, len)
    char *file; /* file name */
    int *len; /* seq len */
{
    char line[1024], *pseq;
    register char *px, *py;
    int natgc, tlen;
    FILE *fp;

    if ((fp = fopen(file, "r")) == 0) {
        fprintf(stderr, "%s: can't read %s\n", prog, file);
        exit(1);
    }
    tlen = natgc = 0;
    while (fgets(line, 1024, fp)) {
        if (*line == ';' || *line == '<' || *line == '>')
            continue;
        for (px = line; *px != '\n'; px++)
            if (isupper(*px) || islower(*px))
                tlen++;
    }
    if ((pseq = malloc((unsigned)(tlen+6))) == 0) {
        fprintf(stderr, "%s: malloc() failed to get %d bytes for %s\n", prog, tlen+6, file);
        exit(1);
    }
    pseq[0] = pseq[1] = pseq[2] = pseq[3] = '\0';

```

cleanup

getseq

Table 1 (cont')

...getseq

```

py = pseq + 4;
*len = tlen;
rewind(fp);

while (fgets(line, 1024, fp)) {
    if (*line == ';' || *line == '<' || *line == '>')
        continue;
    for (px = line; *px != '\n'; px++) {
        if (isupper(*px))
            *py++ = *px;
        else if (islower(*px))
            *py++ = toupper(*px);
        if (index("ATGCU",*(py-1)))
            natgc++;
    }
    *py++ = '\0';
    *py = '\0';
    (void) fclose(fp);
    dna = natgc > (tlen/3);
    return(pseq+4);
}

```

```

char *
g_calloc(msg, nx, sz)
char      *msg;          /* program, calling routine */
int nx, sz;              /* number and size of elements */
{
    char      *px, *calloc();

    if ((px = calloc((unsigned)nx, (unsigned)sz)) == 0) {
        if (*msg) {
            fprintf(stderr, "%s: g_calloc() failed %s (n=%d, sz=%d)\n", prog, msg, nx, sz);
            exit(1);
        }
    }
    return(px);
}

/*
 * get final jmps from dx[] or tmp file, set pp[], reset dmax: main()
 */

```

g_calloc

```

readjmps()
{
    int      fd = -1;
    int      siz, i0, i1;
    register i, j, xx;

    if (fj) {
        (void) fclose(fj);
        if (((fd = open(jname, O_RDONLY, 0)) < 0) {
            fprintf(stderr, "%s: can't open() %s\n", prog, jname);
            cleanup(1);
        }
    }
    for (i = i0 = i1 = 0, dmax0 = dmax, xx = len0; i++) {
        while (1) {
            for (j = dx[dmax].ijmp; j >= 0 && dx[dmax].jp.x[j] >= xx; j--)

```

readjmps

Table 1 (cont')

...readjumps

```

        if (j < 0 && dx[dmax].offset && fj) {
            (void) lseek(fd, dx[dmax].offset, 0);
            (void) read(fd, (char *)&dx[dmax].jp, sizeof(struct jmp));
            (void) read(fd, (char *)&dx[dmax].offset, sizeof(dx[dmax].offset));
            dx[dmax].ijmp = MAXJMP-1;
        }
        else
            break;
    }
    if (i >= JMPS) {
        fprintf(stderr, "%s: too many gaps in alignment\n", prog);
        cleanup(1);
    }
    if (j >= 0) {
        siz = dx[dmax].jp.n[j];
        xx = dx[dmax].jp.x[j];
        dmax += siz;
        if (siz < 0) { /* gap in second seq */
            pp[1].n[i1] = -siz;
            xx += siz;
            /* id = xx - yy + len1 - 1
             */
            pp[1].x[i1] = xx - dmax + len1 - 1;
            gapy++;
            ngapy -= siz;
        }
        /* ignore MAXGAP when doing endgaps */
        siz = (-siz < MAXGAP || endgaps)? -siz : MAXGAP;
        i1++;
    }
    else if (siz > 0) { /* gap in first seq */
        pp[0].n[i0] = siz;
        pp[0].x[i0] = xx;
        gapx++;
        ngapx += siz;
        /* ignore MAXGAP when doing endgaps */
        siz = (siz < MAXGAP || endgaps)? siz : MAXGAP;
        i0++;
    }
}
else
    break;
}

/* reverse the order of jumps
 */
for (j = 0, i0--; j < i0; j++, i0--) {
    i = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0].n[i0] = i;
    i = pp[0].x[j]; pp[0].x[j] = pp[0].x[i0]; pp[0].x[i0] = i;
}
for (j = 0, i1--; j < i1; j++, i1--) {
    i = pp[1].n[j]; pp[1].n[j] = pp[1].n[i1]; pp[1].n[i1] = i;
    i = pp[1].x[j]; pp[1].x[j] = pp[1].x[i1]; pp[1].x[i1] = i;
}
if (fd >= 0)
    (void) close(fd);
if (fj) {
    (void) unlink(jname);
    fj = 0;
    offset = 0;
}
}

```

Table 1 (cont')

```

/*
 * write a filled jmp struct offset of the prev one (if any): nw()
 */
writejumps(ix)                                     writejumps
{
    int ix;

    char      *mktemp();

    if (!fj) {
        if (mktemp(jname) < 0) {
            fprintf(stderr, "%s: can't mktemp() %s\n", prog, jname);
            cleanup(1);
        }
        if ((fj = fopen(jname, "w")) == 0) {
            fprintf(stderr, "%s: can't write %s\n", prog, jname);
            exit(1);
        }
    }
    (void) fwrite((char *)&dx[ix].jp, sizeof(struct jmp), 1, fj);
    (void) fwrite((char *)&dx[ix].offset, sizeof(dx[ix].offset), 1, fj);
}

```

Table 2A

PRO	XXXXXXXXXXXXXXXXX	(Length = 15 amino acids)
Comparison Protein	XXXXXXYYYYYYY	(Length = 12 amino acids)

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

5 divided by 15 = 33.3%

Table 2B

PRO	XXXXXXXXXX	(Length = 10 amino acids)
Comparison Protein	XXXXXXYYYYYYZZYZ	(Length = 15 amino acids)

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

5 divided by 10 = 50%

Table 2C

PRO-DNA	NNNNNNNNNNNNNNNN	(Length = 14 nucleotides)
Comparison DNA	NNNNNNLLLLLLLLLL	(Length = 16 nucleotides)

% nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

6 divided by 14 = 42.9%

Table 2D

PRO-DNA	NNNNNNNNNNNN	(Length = 12 nucleotides)
Comparison DNA	NNNNLLL	(Length = 9 nucleotides)

% nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

4 divided by 12 = 33.3%

II. Compositions and Methods of the Invention

A. PRO Variants

In addition to the full-length native sequence PRO polypeptides described herein, it is contemplated that PRO variants can be prepared. PRO variants can be prepared by introducing appropriate nucleotide changes into the PRO DNA, and/or by synthesis of the desired PRO polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the PRO polypeptide such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

Variations in the native full-length sequence PRO polypeptide or in various domains of the PRO polypeptide described herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the PRO polypeptide that results in a change in the amino acid sequence of the PRO polypeptide as compared with the native sequence PRO polypeptide. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the PRO polypeptide. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the PRO polypeptide with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *i.e.*, conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

In particular embodiments, conservative substitutions of interest are shown in Table 3 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 3, or as further described below in reference to amino acid classes, are introduced and the products screened.

Table 3

	Original Residue	Exemplary Substitutions	Preferred Substitutions
	Ala (A)	val; leu; ile	val
5	Arg (R)	lys; gln; asn	lys
	Asn (N)	gln; his; lys; arg	gln
	Asp (D)	glu	glu
	Cys (C)	ser	ser
	Gln (Q)	asn	asn
10	Glu (E)	asp	asp
	Gly (G)	pro; ala	ala
	His (H)	asn; gln; lys; arg	arg
	Ile (I)	leu; val; met; ala; phe; norleucine	leu
15	Leu (L)	norleucine; ile; val; met; ala; phe	ile
	Lys (K)	arg; gln; asn	arg
	Met (M)	leu; phe; ile	leu
	Phe (F)	leu; val; ile; ala; tyr	leu
20	Pro (P)	ala	ala
	Ser (S)	thr	thr
	Thr (T)	ser	ser
	Trp (W)	tyr; phe	tyr
	Tyr (Y)	trp; phe; thr; ser	phe
25	Val (V)	ile; leu; met; phe; ala; norleucine	leu

Substantial modifications in function or immunological identity of the PRO polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- (1) hydrophobic: norleucine, met, ala, val, leu, ile;
- (2) neutral hydrophilic: cys, ser, thr;
- (3) acidic: asp, glu;
- 35 (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter *et al.*, Nucl. Acids Res., 13:4331 (1986); Zoller *et al.*, Nucl. Acids Res., 10:6487 (1987)], cassette mutagenesis [Wells *et al.*, Gene, 34:315 (1985)], restriction selection mutagenesis [Wells *et al.*, Philos. Trans. R. Soc. London SerA, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the PRO variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant [Cunningham and Wells, Science, 244: 1081-1085 (1989)]. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, The Proteins, (W.H. Freeman & Co., N.Y.); Chothia, J. Mol. Biol., 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

B. Modifications of PRO Polypeptides

Covalent modifications of PRO polypeptides are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a PRO polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the PRO polypeptide. Derivatization with bifunctional agents is useful, for instance, for crosslinking the PRO polypeptide to a water-insoluble support matrix or surface for use in the method for purifying anti-PRO antibodies, and vice-versa. Commonly used crosslinking agents include, *e.g.*, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propioimide.

Other modifications include deamidation of glutamyl and asparagyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the PRO polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in the native sequence PRO polypeptide (either by removing the underlying glycosylation site or by deleting the glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the native sequence PRO polypeptide. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins,

involving a change in the nature and proportions of the various carbohydrate moieties present.

Addition of glycosylation sites to the PRO polypeptide may be accomplished by altering the amino acid sequence. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence PRO polypeptide (for O-linked glycosylation sites). The PRO amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the PRO polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the PRO polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

Removal of carbohydrate moieties present on the PRO polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, *et al.*, Arch. Biochem. Biophys., 259:52 (1987) and by Edge *et al.*, Anal. Biochem., 118:131 (1981).

Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, Meth. Enzymol., 138:350 (1987).

Another type of covalent modification of the PRO polypeptide comprises linking the PRO polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The PRO polypeptide of the present invention may also be modified in a way to form a chimeric molecule comprising the PRO polypeptide fused to another, heterologous polypeptide or amino acid sequence.

In one embodiment, such a chimeric molecule comprises a fusion of the PRO polypeptide with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the PRO polypeptide. The presence of such epitope-tagged forms of the PRO polypeptide can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the PRO polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-His) or poly-histidine-glycine (poly-His-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field *et al.*, Mol. Cell. Biol., 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan *et al.*, Molecular and Cellular Biology, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky *et al.*, Protein Engineering, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp *et al.*, BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin *et al.*, Science, 255:192-194 (1992)]; an α -tubulin epitope peptide [Skinner *et al.*, J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth *et al.*, Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)].

In an alternative embodiment, the chimeric molecule may comprise a fusion of the PRO polypeptide with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also

referred to as an "immunoadhesin"), such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble (transmembrane domain deleted or inactivated) form of a PRO polypeptide in place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions *see* also, U.S. Patent No. 5,428,130 issued June 27, 1995.

C. Preparation of the PRO Polypeptide

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO polypeptides. In particular, cDNAs encoding PRO polypeptides have been identified and isolated, as disclosed in further detail in the Examples below. It is noted that proteins produced in separate expression rounds may be given different PRO numbers but the UNQ number is unique for any given DNA and the encoded protein, and will not be changed. However, for sake of simplicity, in the present specification the protein encoded by the PRO DNA as well as all further native homologues and variants included in the foregoing definition of PRO polypeptides, will be referred to as "PRO" regardless of their origin or mode of preparation.

The description below relates primarily to production of PRO polypeptides by culturing cells transformed or transfected with a vector containing nucleic acid encoding PRO polypeptides. It is, of course, contemplated that alternative methods that are well known in the art may be employed to prepare the PRO polypeptide. For instance, the PRO polypeptide sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques. *See, e.g., Stewart et al., Solid-Phase Peptide Synthesis* (W.H. Freeman Co.: San Francisco, CA, 1969); Merrifield, *J. Am. Chem. Soc.*, **85**: 2149-2154 (1963). *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, with an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the PRO polypeptide may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length PRO polypeptide.

i. Isolation of DNA Encoding PRO Polypeptides

DNA encoding the PRO polypeptide may be obtained from a cDNA library prepared from tissue believed to possess the mRNA encoding the PRO polypeptide and to express it at a detectable level. Accordingly, DNAs encoding the human PRO polypeptide can be conveniently obtained from cDNA libraries prepared from human tissues, such as described in the Examples. The gene encoding the PRO polypeptide may also be obtained from a genomic library or by oligonucleotide synthesis.

Libraries can be screened with probes (such as antibodies to the PRO polypeptide or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook *et al., supra*. An alternative means to isolate the gene encoding the PRO polypeptide is to use PCR methodology. Sambrook *et al., supra*; Dieffenbach *et al., PCR Primer: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press, 1995).

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like ³²P-labeled ATP, biotinylation, or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook *et al.*, *supra*.

Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined through sequence alignment using computer software programs such as ALIGN, DNASTar, and INHERIT, which employ various algorithms to measure homology.

Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook *et al.*, *supra*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

ii. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for PRO polypeptide production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH, and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in Mammalian Cell Biotechnology: A Practical Approach, M. Butler, ed. (IRL Press, 1991) and Sambrook *et al.*, *supra*.

Methods of transfection are known to the ordinarily skilled artisan, for example, CaPO₄ treatment and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook *et al.*, *supra*, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, **23**: 315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, **52**:456-457 (1978) can be employed. General aspects of mammalian cell host system transformations have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., **130**: 946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), **76**: 3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene or polyornithine, may also be used. For various techniques for transforming mammalian cells, *see*, Keown *et al.*, Methods in Enzymology, **185**: 527-537 (1990) and Mansour *et al.*, Nature, **336**: 348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include, but are not limited to, eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325); and K5 772 (ATCC 53,635). Other suitable prokaryotic host cells include Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA*; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan'*; *E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kan'*; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990. Alternatively, *in vitro* methods of cloning, e.g., PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for vectors encoding the PRO polypeptide. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nurse, Nature, 290: 140 [1981]; EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer *et al.*, Bio/Technology, 9: 968-975 (1991)) such as, e.g., *K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt *et al.*, J. Bacteriol., 737 [1983]), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilum* (ATCC 36,906; Van den Berg *et al.*, Bio/Technology, 8: 135 (1990)), *K. thermotolerans*, and *K. marxianus*; *yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna *et al.*, J. Basic Microbiol., 28: 265-278 [1988]); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case *et al.*, Proc. Natl. Acad. Sci. USA, 76: 5259-5263 [1979]); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance *et al.*, Biochem. Biophys. Res. Commun., 112: 284-289 [1983]; Tilburn *et al.*, Gene, 26: 205-221 [1983]; Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81: 1470-1474 [1984]) and *A. niger* (Kelly and Hynes, EMBO J., 4: 475-479 [1985]). Methylophilic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, The Biochemistry of Methylophilic Yeasts, 269 (1982).

Suitable host cells for the expression of nucleic acid encoding glycosylated PRO polypeptides are derived from

multicellular organisms: Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen. Virol., 36: 59 (1977)); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

iii. Selection and Use of a Replicable Vector

The nucleic acid (*e.g.*, cDNA or genomic DNA) encoding the PRO polypeptide may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence if the sequence is to be secreted, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques that are known to the skilled artisan.

The PRO polypeptide may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the DNA encoding the PRO polypeptide that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α -factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV, or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients

not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the nucleic acid encoding the PRO polypeptide such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub *et al.*, Proc. Natl. Acad. Sci. USA, 77: 4216 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7. Stinchcomb *et al.*, Nature, 282: 39 (1979); Kingsman *et al.*, Gene, 7: 141 (1979); Tschemper *et al.*, Gene, 10: 157 (1980). The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, Genetics, 85: 12 (1977).

Expression and cloning vectors usually contain a promoter operably linked to the nucleic acid sequence encoding the PRO polypeptide to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the β -lactamase and lactose promoter systems (Chang *et al.*, Nature, 275: 615 (1978); Goeddel *et al.*, Nature, 281: 544 (1979)), alkaline phosphatase, a tryptophan (*trp*) promoter system (Goeddel, Nucleic Acids Res., 8: 4057 (1980); EP 36,776), and hybrid promoters such as the *tac* promoter (deBoer *et al.*, Proc. Natl. Acad. Sci. USA, 80: 21-25 (1983)). Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the PRO polypeptide.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255: 2073 (1980)) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg., 7: 149 (1968); Holland, Biochemistry, 17: 4900 (1978)), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters that are inducible promoters having the additional advantage of transcription controlled by growth conditions are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

PRO nucleic acid transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus, and Simian Virus 40 (SV40); by heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter; and by heat-shock promoters, provided such promoters are compatible with the host cell systems.

Transcription of a DNA encoding the PRO polypeptide by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin). Typically, however, one will use an enhancer from

a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the sequence coding for PRO polypeptides, but is preferably located at a site 5' from the promoter.

5 Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding the PRO polypeptide.

10 Still other methods, vectors, and host cells suitable for adaptation to the synthesis of the PRO polypeptide in recombinant vertebrate cell culture are described in Gething *et al.*, Nature, 293: 620-625 (1981); Mantei *et al.*, Nature, 281: 40-46 (1979); EP 117,060; and EP 117,058.

iv. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional.
15 Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 (1980)), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so
20 that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared
25 against a native-sequence PRO polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to DNA encoding the PRO polypeptide and encoding a specific antibody epitope.

v. Purification of PRO Polypeptides

Forms of PRO polypeptides may be recovered from culture medium or from host cell lysates. If membrane-
30 bound, it can be released from the membrane using a suitable detergent solution (e.g., TRITON-X™ 100) or by enzymatic cleavage. Cells employed in expression of nucleic acid encoding the PRO polypeptide can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell-lysing agents. It may be desired to purify the PRO polypeptide from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange
35 column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example,

Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the PRO polypeptide. Various methods of protein purification may be employed and such methods are known in the art and described, for example, in Deutscher, Methods in Enzymology, 182 (1990); Scopes, Protein Purification: Principles and Practice (Springer-Verlag: New York, 1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular PRO polypeptide produced.

D. Uses of PRO Polypeptides

i. Assays for Cardiovascular, Endothelial, and Angiogenic Activity

Various assays can be used to test the polypeptide herein for cardiovascular, endothelial, and angiogenic activity. Such assays include those provided in the Examples below.

Assays for testing for endothelin antagonist activity, as disclosed in U.S. Pat. No. 5,773,414, include a rat heart ventricle binding assay where the polypeptide is tested for its ability to inhibit iodinated endothelin-1 binding in a receptor assay, an endothelin receptor binding assay testing for intact cell binding of radiolabeled endothelin-1 using rabbit renal artery vascular smooth muscle cells, an inositol phosphate accumulation assay where functional activity is determined in Rat-1 cells by measuring intra-cellular levels of second messengers, an arachidonic acid release assay that measures the ability of added compounds to reduce endothelin-stimulated arachidonic acid release in cultured vascular smooth muscles, *in vitro* (isolated vessel) studies using endothelium from male New Zealand rabbits, and *in vivo* studies using male Sprague-Dawley rats.

Assays for tissue generation activity include, without limitation, those described in WO 95/16035 (bone, cartilage, tendon); WO 95/05846 (nerve, neuronal), and WO 91/07491 (skin, endothelium).

Assays for wound-healing activity include, for example, those described in Winter, Epidermal Wound Healing, Maibach, HI and Rovee, DT, eds. (Year Book Medical Publishers, Inc., Chicago), pp. 71-112, as modified by the article of Eaglstein and Mertz, J. Invest. Dermatol., 71: 382-384 (1978).

An assay to screen for a test molecule relating to a PRO polypeptide that binds an endothelin B₁ (ETB₁) receptor polypeptide and modulates signal transduction activity involves providing a host cell transformed with a DNA encoding endothelin B₁ receptor polypeptide, exposing the cells to the test candidate, and measuring endothelin B₁ receptor signal transduction activity, as described, *e.g.*, in U.S. Pat. No. 5,773,223.

There are several cardiac hypertrophy assays. *In vitro* assays include induction of spreading of adult rat cardiac myocytes. In this assay, ventricular myocytes are isolated from a single (male Sprague-Dawley) rat, essentially following a modification of the procedure described in detail by Piper *et al.*, "Adult ventricular rat heart muscle cells" in Cell Culture Techniques in Heart and Vessel Research, H.M. Piper, ed. (Berlin: Springer-Verlag, 1990), pp. 36-60. This procedure permits the isolation of adult ventricular myocytes and the long-term culture of these cells in the rod-shaped phenotype. Phenylephrine and Prostaglandin F_{2α} (PGF_{2α}) have been shown to induce a spreading response in these adult cells. The inhibition of myocyte spreading induced by PGF_{2α} or PGF_{2α} analogs (*e.g.*, fluprostenol) and phenylephrine by various potential inhibitors of cardiac hypertrophy is then tested.

One example of an *in vivo* assay is a test for inhibiting cardiac hypertrophy induced by fluprostenol *in vivo*. This pharmacological model tests the ability of the PRO polypeptide to inhibit cardiac hypertrophy induced in rats (*e.g.*, male Wistar or Sprague-Dawley) by subcutaneous injection of fluprostenol (an agonist analog of PGF_{2α}). It is known that rats with pathologic cardiac hypertrophy induced by myocardial infarction have chronically elevated levels of extractable PGF_{2α} in their myocardium. Lai *et al.*, Am. J. Physiol. (Heart Circ. Physiol.), **271**: H2197-H2208 (1996). Accordingly, factors that can inhibit the effects of fluprostenol on myocardial growth *in vivo* are potentially useful for treating cardiac hypertrophy. The effects of the PRO polypeptide on cardiac hypertrophy are determined by measuring the weight of heart, ventricles, and left ventricle (normalized by body weight) relative to fluprostenol-treated rats not receiving the PRO polypeptide.

Another example of an *in vivo* assay is the pressure-overload cardiac hypertrophy assay. For *in vivo* testing it is common to induce pressure-overload cardiac hypertrophy by constriction of the abdominal aorta of test animals. In a typical protocol, rats (*e.g.*, male Wistar or Sprague-Dawley) are treated under anesthesia, and the abdominal aorta of each rat is narrowed down just below the diaphragm. Beznak M., Can. J. Biochem. Physiol., **33**: 985-94 (1955). The aorta is exposed through a surgical incision, and a blunted needle is placed next to the vessel. The aorta is constricted with a ligature of silk thread around the needle, which is immediately removed and which reduces the lumen of the aorta to the diameter of the needle. This approach is described, for example, in Rossi *et al.*, Am. Heart J., **124**: 700-709 (1992) and O'Rourke and Reibel, P.S.E.M.B., **200**: 95-100 (1992).

In yet another *in vivo* assay, the effect on cardiac hypertrophy following experimentally induced myocardial infarction (MI) is measured. Acute MI is induced in rats by left coronary artery ligation and confirmed by electrocardiographic examination. A sham-operated group of animals is also prepared as control animals. Earlier data have shown that cardiac hypertrophy is present in the group of animals with MI, as evidenced by an 18% increase in heart weight-to-body weight ratio. Lai *et al.*, *supra*. Treatment of these animals with candidate blockers of cardiac hypertrophy, *e.g.*, the PRO polypeptide, provides valuable information about the therapeutic potential of the candidates tested. One further such assay test for induction of cardiac hypertrophy is disclosed in U.S. Pat. No. 5,773,415, using Sprague-Dawley rats.

For cancer, a variety of well-known animal models can be used to further understand the role of the genes identified herein in the development and pathogenesis of tumors, and to test the efficacy of candidate therapeutic agents, including antibodies and other antagonists of native PRO polypeptides, such as small-molecule antagonists. The *in vivo* nature of such models makes them particularly predictive of responses in human patients. Animal models of tumors and cancers (*e.g.*, breast cancer, colon cancer, prostate cancer, lung cancer, etc.) include both non-recombinant and recombinant (transgenic) animals. Non-recombinant animal models include, for example, rodent, *e.g.*, murine models. Such models can be generated by introducing tumor cells into syngeneic mice using standard techniques, *e.g.*, subcutaneous injection, tail vein injection, spleen implantation, intraperitoneal implantation, implantation under the renal capsule, or orthotopic implantation, *e.g.*, colon cancer cells implanted in colonic tissue. See, *e.g.*, PCT publication No. WO 97/33551, published September 18, 1997. Probably the most often used animal species in oncological studies are immunodeficient mice and, in particular, nude mice. The observation that the nude mouse with thymic hypo/aplasia could successfully act as a host for human tumor xenografts has lead to its widespread use for this purpose. The autosomal recessive *nu* gene has been introduced

into a very large number of distinct congenic strains of nude mouse, including, for example, ASW, A/He, AKR, BALB/c, B10.LP, C17, C3H, C57BL, C57, CBA, DBA, DDD, I/St, NC, NFR, NFS, NFS/N, NZB, NZC, NZW, P, RIII, and SJL. In addition, a wide variety of other animals with inherited immunological defects other than the nude mouse have been bred and used as recipients of tumor xenografts. For further details *see, e.g., The Nude*

5 Mouse in Oncology Research, E. Boven and B. Winograd, eds. (CRC Press, Inc., 1991).

The cells introduced into such animals can be derived from known tumor/cancer cell lines, such as any of the above-listed tumor cell lines, and, for example, the B104-1-1 cell line (stable NIH-3T3 cell line transfected with the *neu* protooncogene); *ras*-transfected NIH-3T3 cells; Caco-2 (ATCC HTB-37); or a moderately well-differentiated grade II human colon adenocarcinoma cell line, HT-29 (ATCC HTB-38); or from tumors and cancers.

10 Samples of tumor or cancer cells can be obtained from patients undergoing surgery, using standard conditions involving freezing and storing in liquid nitrogen. Karmali *et al.*, Br. J. Cancer, 48: 689-696 (1983).

Tumor cells can be introduced into animals such as nude mice by a variety of procedures. The subcutaneous (s.c.) space in mice is very suitable for tumor implantation. Tumors can be transplanted s.c. as solid blocks, as needle biopsies by use of a trochar, or as cell suspensions. For solid-block or trochar implantation, tumor tissue fragments of suitable size are introduced into the s.c. space. Cell suspensions are freshly prepared from primary tumors or stable tumor cell lines, and injected subcutaneously. Tumor cells can also be injected as subdermal implants. In this location, the inoculum is deposited between the lower part of the dermal connective tissue and the s.c. tissue.

20 Animal models of breast cancer can be generated, for example, by implanting rat neuroblastoma cells (from which the *neu* oncogene was initially isolated), or *neu*-transformed NIH-3T3 cells into nude mice, essentially as described by Drebin *et al.* Proc. Nat. Acad. Sci. USA, 83: 9129-9133 (1986).

Similarly, animal models of colon cancer can be generated by passaging colon cancer cells in animals, *e.g.*, nude mice, leading to the appearance of tumors in these animals. An orthotopic transplant model of human colon cancer in nude mice has been described, for example, by Wang *et al.*, Cancer Research, 54: 4726-4728 (1994) and Too *et al.*, Cancer Research, 55: 681-684 (1995). This model is based on the so-called "METAMOUSE™" sold by AntiCancer, Inc., (San Diego, California).

30 Tumors that arise in animals can be removed and cultured *in vitro*. Cells from the *in vitro* cultures can then be passaged to animals. Such tumors can serve as targets for further testing or drug screening. Alternatively, the tumors resulting from the passage can be isolated and RNA from pre-passage cells and cells isolated after one or more rounds of passage analyzed for differential expression of genes of interest. Such passaging techniques can be performed with any known tumor or cancer cell lines.

For example, Meth A, CMS4, CMS5, CMS21, and WEHI-164 are chemically induced fibrosarcomas of BALB/c female mice (DeLeo *et al.*, J. Exp. Med., 146: 720 (1977)), which provide a highly controllable model system for studying the anti-tumor activities of various agents. Palladino *et al.*, J. Immunol., 138: 4023-4032 (1987). Briefly, tumor cells are propagated *in vitro* in cell culture. Prior to injection into the animals, the cell lines are washed and suspended in buffer, at a cell density of about 10×10^6 to 10×10^7 cells/ml. The animals are then infected subcutaneously with 10 to 100 μ l of the cell suspension, allowing one to three weeks for a tumor to appear.

In addition, the Lewis lung (3LL) carcinoma of mice, which is one of the most thoroughly studied

experimental tumors. can be used as an investigational tumor model. Efficacy in this tumor model has been correlated with beneficial effects in the treatment of human patients diagnosed with small-cell carcinoma of the lung (SCCL). This tumor can be introduced in normal mice upon injection of tumor fragments from an affected mouse or of cells maintained in culture. Zupi *et al.*, Br. J. Cancer, 41: suppl. 4, 30 (1980). Evidence indicates that tumors
5 can be started from injection of even a single cell and that a very high proportion of infected tumor cells survive. For further information about this tumor model see, Zacharski, Haemostasis, 16: 300-320 (1986).

One way of evaluating the efficacy of a test compound in an animal model with an implanted tumor is to measure the size of the tumor before and after treatment. Traditionally, the size of implanted tumors has been measured with a slide caliper in two or three dimensions. The measure limited to two dimensions does not
10 accurately reflect the size of the tumor; therefore, it is usually converted into the corresponding volume by using a mathematical formula. However, the measurement of tumor size is very inaccurate. The therapeutic effects of a drug candidate can be better described as treatment-induced growth delay and specific growth delay. Another important variable in the description of tumor growth is the tumor volume doubling time. Computer programs for the calculation and description of tumor growth are also available, such as the program reported by Rygaard and
15 Spang-Thomsen, Proc. 6th Int. Workshop on Immune-Deficient Animals, Wu and Sheng eds. (Basel, 1989), p. 301. It is noted, however, that necrosis and inflammatory responses following treatment may actually result in an increase in tumor size, at least initially. Therefore, these changes need to be carefully monitored, by a combination of a morphometric method and flow cytometric analysis.

Further, recombinant (transgenic) animal models can be engineered by introducing the coding portion of the
20 PRO gene identified herein into the genome of animals of interest, using standard techniques for producing transgenic animals. Animals that can serve as a target for transgenic manipulation include, without limitation, mice, rats, rabbits, guinea pigs, sheep, goats, pigs, and non-human primates, *e.g.*, baboons, chimpanzees and monkeys. Techniques known in the art to introduce a transgene into such animals include pronucleic microinjection (U.S. Patent No. 4,873,191); retrovirus-mediated gene transfer into germ lines (*e.g.*, Van der Putten *et al.*, Proc. Natl.
25 Acad. Sci. USA, 82: 6148-615 (1985)); gene targeting in embryonic stem cells (Thompson *et al.*, Cell, 56: 313-321 (1989)); electroporation of embryos (Lo, Mol. Cell. Biol., 3: 1803-1814 (1983)); and sperm-mediated gene transfer. Lavitrano *et al.*, Cell, 57: 717-73 (1989). For a review, see for example, U.S. Patent No. 4,736,866.

For the purpose of the present invention, transgenic animals include those that carry the transgene only in part of their cells ("mosaic animals"). The transgene can be integrated either as a single transgene, or in concatamers,
30 *e.g.*, head-to-head or head-to-tail tandems. Selective introduction of a transgene into a particular cell type is also possible by following, for example, the technique of Lasko *et al.*, Proc. Natl. Acad. Sci. USA, 89: 6232-636 (1992).

The expression of the transgene in transgenic animals can be monitored by standard techniques. For example, Southern blot analysis or PCR amplification can be used to verify the integration of the transgene. The level of mRNA expression can then be analyzed using techniques such as *in situ* hybridization, Northern blot analysis, PCR,
35 or immunocytochemistry. The animals are further examined for signs of tumor or cancer development.

Alternatively, "knock-out" animals can be constructed that have a defective or altered gene encoding a PRO polypeptide identified herein, as a result of homologous recombination between the endogenous gene encoding the PRO polypeptide and altered genomic DNA encoding the same polypeptide introduced into an embryonic cell of

the animal. For example, cDNA encoding a particular PRO polypeptide can be used to clone genomic DNA encoding that polypeptide in accordance with established techniques. A portion of the genomic DNA encoding a particular PRO polypeptide can be deleted or replaced with another gene, such as a gene encoding a selectable marker that can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector. *See, e.g.,* Thomas and Capecchi, Cell, 51: 503 (1987) for a description of homologous recombination vectors. The vector is introduced into an embryonic stem cell line (*e.g.,* by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected. *See, e.g.,* Li *et al.*, Cell, 69: 915 (1992). The selected cells are then injected into a blastocyst of an animal (*e.g.,* a mouse or rat) to form aggregation chimeras. *See, e.g.,* Bradley, in Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, E. J. Robertson, ed. (IRL: Oxford, 1987), pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock-out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized, for instance, by their ability to defend against certain pathological conditions and by their development of pathological conditions due to absence of the PRO polypeptide.

The efficacy of antibodies specifically binding the PRO polypeptides identified herein, and other drug candidates, can be tested also in the treatment of spontaneous animal tumors. A suitable target for such studies is the feline oral squamous cell carcinoma (SCC). Feline oral SCC is a highly invasive, malignant tumor that is the most common oral malignancy of cats, accounting for over 60% of the oral tumors reported in this species. It rarely metastasizes to distant sites, although this low incidence of metastasis may merely be a reflection of the short survival times for cats with this tumor. These tumors are usually not amenable to surgery, primarily because of the anatomy of the feline oral cavity. At present, there is no effective treatment for this tumor. Prior to entry into the study, each cat undergoes complete clinical examination and biopsy, and is scanned by computed tomography (CT). Cats diagnosed with sublingual oral squamous cell tumors are excluded from the study. The tongue can become paralyzed as a result of such tumor, and even if the treatment kills the tumor, the animals may not be able to feed themselves. Each cat is treated repeatedly, over a longer period of time. Photographs of the tumors will be taken daily during the treatment period, and at each subsequent recheck. After treatment, each cat undergoes another CT scan. CT scans and thoracic radiograms are evaluated every 8 weeks thereafter. The data are evaluated for differences in survival, response, and toxicity as compared to control groups. Positive response may require evidence of tumor regression, preferably with improvement of quality of life and/or increased life span.

In addition, other spontaneous animal tumors, such as fibrosarcoma, adenocarcinoma, lymphoma, chondroma, or leiomyosarcoma of dogs, cats, and baboons can also be tested. Of these, mammary adenocarcinoma in dogs and cats is a preferred model as its appearance and behavior are very similar to those in humans. However, the use of this model is limited by the rare occurrence of this type of tumor in animals.

Other *in vitro* and *in vivo* cardiovascular, endothelial, and angiogenic tests known in the art are also suitable herein.

ii. Tissue Distribution

The results of the cardiovascular, endothelial, and angiogenic assays herein can be verified by further studies, such as by determining mRNA expression in various human tissues.

As noted before, gene amplification and/or gene expression in various tissues may be measured by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 (1980)), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes.

Gene expression in various tissues, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native-sequence PRO polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to PRO DNA and encoding a specific antibody epitope. General techniques for generating antibodies, and special protocols for *in situ* hybridization are provided hereinbelow.

iii. Antibody Binding Studies

The results of the cardiovascular, endothelial, and angiogenic study can be further verified by antibody binding studies, in which the ability of anti-PRO antibodies to inhibit the effect of the PRO polypeptides on endothelial cells or other cells used in the cardiovascular, endothelial, and angiogenic assays is tested. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies, the preparation of which will be described hereinbelow.

Antibody binding studies may be carried out in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, Monoclonal Antibodies: A Manual of Techniques (CRC Press, Inc., 1987), pp.147-158.

Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of target protein in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies preferably are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte that remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody that is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, e.g., U.S. Pat. No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA

assay, in which case the detectable moiety is an enzyme.

For immunohistochemistry, the tissue sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin, for example.

iv. Cell-Based Tumor Assays

5 Cell-based assays and animal models for cardiovascular, endothelial, and angiogenic disorders, such as tumors, can be used to verify the findings of a cardiovascular, endothelial, and angiogenic assay herein, and further to understand the relationship between the genes identified herein and the development and pathogenesis of undesirable cardiovascular, endothelial, and angiogenic cell growth. The role of gene products identified herein in the development and pathology of undesirable cardiovascular, endothelial, and angiogenic cell growth, *e.g.*, tumor
10 cells, can be tested by using cells or cells lines that have been identified as being stimulated or inhibited by the PRO polypeptide herein. Such cells include, for example, those set forth in the Examples below.

In a different approach, cells of a cell type known to be involved in a particular cardiovascular, endothelial, and angiogenic disorder are transfected with the cDNAs herein, and the ability of these cDNAs to induce excessive growth or inhibit growth is analyzed. If the cardiovascular, endothelial, and angiogenic disorder is cancer, suitable
15 tumor cells include, for example, stable tumor cell lines such as the B104-1-1 cell line (stable NIH-3T3 cell line transfected with the *neu* protooncogene) and *ras*-transfected NIH-3T3 cells, which can be transfected with the desired gene and monitored for tumorigenic growth. Such transfected cell lines can then be used to test the ability of poly- or monoclonal antibodies or antibody compositions to inhibit tumorigenic cell growth by exerting cytostatic or cytotoxic activity on the growth of the transformed cells, or by mediating antibody-dependent cellular cytotoxicity
20 (ADCC). Cells transfected with the coding sequences of the genes identified herein can further be used to identify drug candidates for the treatment of cardiovascular, endothelial, and angiogenic disorders such as cancer.

In addition, primary cultures derived from tumors in transgenic animals (as described above) can be used in the cell-based assays herein, although stable cell lines are preferred. Techniques to derive continuous cell lines from transgenic animals are well known in the art. *See, e.g.*, Small *et al.*, Mol. Cell. Biol., 5: 642-648 (1985).

25 v. Gene Therapy

The PRO polypeptides herein and polypeptidyl agonists and antagonists may be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as gene therapy.

There are two major approaches to getting the nucleic acid (optionally contained in a vector) into the patient's cells: *in vivo* and *ex vivo*. For *in vivo* delivery the nucleic acid is injected directly into the patient, usually at the sites
30 where the PRO polypeptide is required, *i.e.*, the site of synthesis of the PRO polypeptide, if known, and the site (*e.g.*, wound) where biological activity of the PRO polypeptide is needed. For *ex vivo* treatment, the patient's cells are removed, the nucleic acid is introduced into these isolated cells, and the modified cells are administered to the patient either directly or, for example, encapsulated within porous membranes that are implanted into the patient (*see, e.g.*, U.S. Pat. Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing
35 nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or transferred *in vivo* in the cells of the intended host. Techniques suitable for the transfer

of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, transduction, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. Transduction involves the association of a replication-defective, recombinant viral (preferably retroviral) particle with a cellular receptor, followed by introduction of the nucleic acids contained by the particle into the cell. A commonly used vector for *ex vivo* delivery of the gene is a retrovirus.

The currently preferred *in vivo* nucleic acid transfer techniques include transfection with viral or non-viral vectors (such as adenovirus, lentivirus, Herpes simplex I virus, or adeno-associated virus (AAV)) and lipid-based systems (useful lipids for lipid-mediated transfer of the gene are, for example, DOTMA, DOPE, and DC-Chol; *see, e.g.,* Tonkinson *et al.*, Cancer Investigation, 14(1): 54-65 (1996)). The most preferred vectors for use in gene therapy are viruses, most preferably adenoviruses, AAV, lentiviruses, or retroviruses. A viral vector such as a retroviral vector includes at least one transcriptional promoter/enhancer or locus-defining element(s), or other elements that control gene expression by other means such as alternate splicing, nuclear RNA export, or post-translational modification of messenger. In addition, a viral vector such as a retroviral vector includes a nucleic acid molecule that, when transcribed in the presence of a gene encoding the PRO polypeptide, is operably linked thereto and acts as a translation initiation sequence. Such vector constructs also include a packaging signal, long terminal repeats (LTRs) or portions thereof, and positive and negative strand primer binding sites appropriate to the virus used (if these are not already present in the viral vector). In addition, such vector typically includes a signal sequence for secretion of the PRO polypeptide from a host cell in which it is placed. Preferably the signal sequence for this purpose is a mammalian signal sequence, most preferably the native signal sequence for the PRO polypeptide. Optionally, the vector construct may also include a signal that directs polyadenylation, as well as one or more restriction sites and a translation termination sequence. By way of example, such vectors will typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second-strand DNA synthesis, and a 3' LTR or a portion thereof. Other vectors can be used that are non-viral, such as cationic lipids, polylysine, and dendrimers.

In some situations, it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell-surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins that bind to a cell-surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.,* capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins that undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, J. Biol. Chem., 262: 4429-4432 (1987); and Wagner *et al.*, Proc. Natl. Acad. Sci. USA, 87: 3410-3414 (1990). For a review of the currently known gene marking and gene therapy protocols, *see, Anderson et al.*, Science, 256: 808-813 (1992). *See also* WO 93/25673 and the references cited therein.

Suitable gene therapy and methods for making retroviral particles and structural proteins can be found in, *e.g.*, U.S. Pat. No. 5,681,746.

vi. Use of Gene as a Diagnostic

This invention is also related to the use of the gene encoding the PRO polypeptide as a diagnostic. Detection

of a mutated form of the PRO polypeptide will allow a diagnosis of a cardiovascular, endothelial, and angiogenic disease or a susceptibility to a cardiovascular, endothelial, and angiogenic disease, such as a tumor, since mutations in the PRO polypeptide may cause tumors.

5 Individuals carrying mutations in the genes encoding a human PRO polypeptide may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy, and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki *et al.*, Nature, 324: 163-166 (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid encoding the PRO polypeptide can be used to identify and analyze the PRO polypeptide mutations. For example, 10 deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled RNA encoding the PRO polypeptide, or alternatively, radiolabeled antisense DNA sequences encoding the PRO polypeptide. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

15 Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures. See, e.g., 20 Myers *et al.*, Science, 230: 1242 (1985).

Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method, for example, Cotton *et al.*, Proc. Natl. Acad. Sci. USA, 85: 4397-4401 (1985).

25 Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing, or the use of restriction enzymes, e.g., restriction fragment length polymorphisms (RFLP), and Southern blotting of genomic DNA.

vii. Use to Detect PRO Polypeptide Levels

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

30 Expression of nucleic acid encoding the PRO polypeptide may be linked to vascular disease or neovascularization associated with tumor formation. If the PRO polypeptide has a signal sequence and the mRNA is highly expressed in endothelial cells and to a lesser extent in smooth muscle cells, this indicates that the PRO polypeptide is present in serum. Accordingly, an anti-PRO polypeptide antibody could be used to diagnose vascular disease or neovascularization associated with tumor formation, since an altered level of this PRO polypeptide may 35 be indicative of such disorders.

A competition assay may be employed wherein antibodies specific to the PRO polypeptide are attached to a solid support and the labeled PRO polypeptide and a sample derived from the host are passed over the solid support

and the amount of label detected attached to the solid support can be correlated to a quantity of the PRO polypeptide in the sample.

viii. Chromosome Mapping

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis for the 3'- untranslated region is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome-specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 500 or 600 bases; however, clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the clones from which the gene encoding the PRO polypeptide was derived, and the longer the better. For example, 2,000 bp is good, 4,000 bp is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see, Verma *et al.*, Human Chromosomes: a Manual of Basic Techniques (Pergamon Press, New York, 1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available online through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region is then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to

a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

ix. Screening Assays for Drug Candidates

This invention encompasses methods of screening compounds to identify those that mimic the PRO polypeptide (agonists) or prevent the effect of the PRO polypeptide (antagonists). Screening assays for antagonist drug candidates are designed to identify compounds that bind or complex with the PRO polypeptide encoded by the genes identified herein, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates.

The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays, and cell-based assays, which are well characterized in the art.

All assays for antagonists are common in that they call for contacting the drug candidate with a PRO polypeptide encoded by a nucleic acid identified herein under conditions and for a time sufficient to allow these two components to interact.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the PRO polypeptide encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, *e.g.*, on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the PRO polypeptide and drying. Alternatively, an immobilized antibody, *e.g.*, a monoclonal antibody, specific for the PRO polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, *e.g.*, the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, *e.g.*, by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labeled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular PRO polypeptide encoded by a gene identified herein, its interaction with that polypeptide can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, *e.g.*, cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers (Fields and Song, Nature (London), 340: 245-246 (1989); Chien *et al.*, Proc. Natl. Acad. Sci. USA, 88: 9578-9582 (1991)) as disclosed by Chevray and Nathans, Proc. Natl. Acad. Sci. USA, 89: 5789-5793 (1991). Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding domain, the other one functioning as the transcription-activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding

domain of GAL4, and another, in which candidate activating proteins are fused to the activation domain. The expression of a GAL1-*lacZ* reporter gene under control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with a chromogenic substrate for β -galactosidase. A complete kit (MATCHMAKER™) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

Compounds that interfere with the interaction of a gene encoding a PRO polypeptide identified herein and other intra- or extracellular components can be tested as follows: usually a reaction mixture is prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a candidate compound to inhibit binding, the reaction is run in the absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction mixture, to serve as positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described hereinabove. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

If the PRO polypeptide has the ability to stimulate the proliferation of endothelial cells in the presence of the co-mitogen ConA, then one example of a screening method takes advantage of this ability. Specifically, in the proliferation assay, human umbilical vein endothelial cells are obtained and cultured in 96-well flat-bottomed culture plates (Costar, Cambridge, MA) and supplemented with a reaction mixture appropriate for facilitating proliferation of the cells, the mixture containing Con-A (Calbiochem, La Jolla, CA). Con-A and the compound to be screened are added and after incubation at 37°C, cultures are pulsed with ³H-thymidine and harvested onto glass fiber filters (phD; Cambridge Technology, Watertown, MA). Mean ³H-thymidine incorporation (cpm) of triplicate cultures is determined using a liquid scintillation counter (Beckman Instruments, Irvine, CA). Significant ³(H)-thymidine incorporation indicates stimulation of endothelial cell proliferation.

To assay for antagonists, the assay described above is performed; however, in this assay the PRO polypeptide is added along with the compound to be screened and the ability of the compound to inhibit ³(H)thymidine incorporation in the presence of the PRO polypeptide indicates that the compound is an antagonist to the PRO polypeptide. Alternatively, antagonists may be detected by combining the PRO polypeptide and a potential antagonist with membrane-bound PRO polypeptide receptors or recombinant receptors under appropriate conditions for a competitive inhibition assay. The PRO polypeptide can be labeled, such as by radioactivity, such that the number of PRO polypeptide molecules bound to the receptor can be used to determine the effectiveness of the potential antagonist. The gene encoding the receptor can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting. Coligan *et al.*, Current Protocols in Immun., 1(2): Chapter 5 (1991). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the PRO polypeptide and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the PRO polypeptide. Transfected cells that are grown on glass slides are exposed to the labeled PRO polypeptide. The PRO polypeptide can be labeled by a variety of

means including iodination or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an interactive sub-pooling and re-screening process, eventually yielding a single clone that encodes the putative receptor.

5 As an alternative approach for receptor identification, the labeled PRO polypeptide can be photoaffinity-linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE and exposed to X-ray film. The labeled complex containing the receptor can be excised, resolved into peptide fragments, and subjected to protein micro-sequencing. The amino acid sequence obtained from micro-sequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify
10 the gene encoding the putative receptor.

In another assay for antagonists, mammalian cells or a membrane preparation expressing the receptor would be incubated with the labeled PRO polypeptide in the presence of the candidate compound. The ability of the compound to enhance or block this interaction could then be measured.

The compositions useful in the treatment of cardiovascular, endothelial, and angiogenic disorders include,
15 without limitation, antibodies, small organic and inorganic molecules, peptides, phosphopeptides, antisense and ribozyme molecules, triple-helix molecules, etc., that inhibit the expression and/or activity of the target gene product.

More specific examples of potential antagonists include an oligonucleotide that binds to the fusions of immunoglobulin with a PRO polypeptide, and, in particular, antibodies including, without limitation, poly- and
20 monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. Alternatively, a potential antagonist may be a closely related protein, for example, a mutated form of the PRO polypeptide that recognizes the receptor but imparts no effect, thereby competitively inhibiting the action of the PRO polypeptide.

25 Another potential PRO polypeptide antagonist or agonist is an antisense RNA or DNA construct prepared using antisense technology, where, *e.g.*, an antisense RNA or DNA molecule acts to block directly the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide
30 sequence, which encodes the mature PRO polypeptides herein, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix - *see, Lee et al., Nucl. Acids Res.*, **6**:3073 (1979); Cooney *et al., Science*, **241**: 456 (1988); Dervan *et al., Science*, **251**:1360 (1991)), thereby preventing transcription and the production of the PRO polypeptide. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks
35 translation of the mRNA molecule into the PRO polypeptide (antisense - Okano, *Neurochem.*, **56**:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression (CRC Press: Boca Raton, FL, 1988). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the PRO polypeptide. When antisense DNA is used,

oligodeoxyribonucleotides derived from the translation-initiation site, e.g., between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.

Antisense RNA or DNA molecules are generally at least about 5 bases in length, about 10 bases in length, about 15 bases in length, about 20 bases in length, about 25 bases in length, about 30 bases in length, about 35 bases in length, about 40 bases in length, about 45 bases in length, about 50 bases in length, about 55 bases in length, about 60 bases in length, about 65 bases in length, about 70 bases in length, about 75 bases in length, about 80 bases in length, about 85 bases in length, about 90 bases in length, about 95 bases in length, about 100 bases in length, or more.

Potential antagonists include small molecules that bind to the active site, the receptor binding site, or growth factor or other relevant binding site of the PRO polypeptide, thereby blocking the normal biological activity of the PRO polypeptide. Examples of small molecules include, but are not limited to, small peptides or peptide-like molecules, preferably soluble peptides, and synthetic non-peptidyl organic or inorganic compounds.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details *see, e.g.*, Rossi, Current Biology, 4: 469-471 (1994), and PCT publication No. WO 97/33551 (published September 18, 1997).

Nucleic acid molecules in triple-helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple-helix formation via Hoogsteen base-pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details *see, e.g.*, PCT publication No. WO 97/33551, *supra*.

These small molecules can be identified by any one or more of the screening assays discussed hereinabove and/or by any other screening techniques well known for those skilled in the art.

x. Types of Cardiovascular, Endothelial, and Angiogenic Disorders to be Treated

The PRO polypeptides, or agonists or antagonists thereto, that have activity in the cardiovascular, angiogenic, and endothelial assays described herein, and/or whose gene product has been found to be localized to the cardiovascular system, are likely to have therapeutic uses in a variety of cardiovascular, endothelial, and angiogenic disorders, including systemic disorders that affect vessels, such as diabetes mellitus. Their therapeutic utility could include diseases of the arteries, capillaries, veins, and/or lymphatics. Examples of treatments hereunder include treating muscle wasting disease, treating osteoporosis, aiding in implant fixation to stimulate the growth of cells around the implant and therefore facilitate its attachment to its intended site, increasing IGF stability in tissues or in serum, if applicable, and increasing binding to the IGF receptor (since IGF has been shown *in vitro* to enhance human marrow erythroid and granulocytic progenitor cell growth).

The PRO polypeptides or agonists or antagonists thereto may also be employed to stimulate erythropoiesis or granulopoiesis, to stimulate wound healing or tissue regeneration and associated therapies concerned with growth of tissue, such as connective tissue, skin, bone, cartilage, muscle, lung, or kidney, to promote angiogenesis, to stimulate or inhibit migration of endothelial cells, and to proliferate the growth of vascular smooth muscle and

endothelial cell production. The increase in angiogenesis mediated by the PRO polypeptide or antagonist would be beneficial to ischemic tissues and to collateral coronary development in the heart subsequent to coronary stenosis. Antagonists are used to inhibit the action of such polypeptides, for example, to limit the production of excess connective tissue during wound healing or pulmonary fibrosis if the PRO polypeptide promotes such production.

5 This would include treatment of acute myocardial infarction and heart failure.

Moreover, the present invention concerns the treatment of cardiac hypertrophy, regardless of the underlying cause, by administering a therapeutically effective dose of the PRO polypeptide, or agonist or antagonist thereto. If the objective is the treatment of human patients, the PRO polypeptide preferably is recombinant human PRO polypeptide (rhPRO polypeptide). The treatment for cardiac hypertrophy can be performed at any of its various stages, which may result from a variety of diverse pathologic conditions, including myocardial infarction, hypertension, hypertrophic cardiomyopathy, and valvular regurgitation. The treatment extends to all stages of the progression of cardiac hypertrophy, with or without structural damage of the heart muscle, regardless of the underlying cardiac disorder.

The decision of whether to use the molecule itself or an agonist thereof for any particular indication, as opposed to an antagonist to the molecule, would depend mainly on whether the molecule herein promotes cardiovascularization, genesis of endothelial cells, or angiogenesis or inhibits these conditions. For example, if the molecule promotes angiogenesis, an antagonist thereof would be useful for treatment of disorders where it is desired to limit or prevent angiogenesis. Examples of such disorders include vascular tumors such as haemangioma, tumor angiogenesis, neovascularization in the retina, choroid, or cornea, associated with diabetic retinopathy or premature infant retinopathy or macular degeneration and proliferative vitreoretinopathy, rheumatoid arthritis, Crohn's disease, atherosclerosis, ovarian hyperstimulation, psoriasis, endometriosis associated with neovascularization, restenosis subsequent to balloon angioplasty, scar tissue overproduction, for example, that seen in a keloid that forms after surgery, fibrosis after myocardial infarction, or fibrotic lesions associated with pulmonary fibrosis.

If, however, the molecule inhibits angiogenesis, it would be expected to be used directly for treatment of the above conditions.

On the other hand, if the molecule stimulates angiogenesis it would be used itself (or an agonist thereof) for indications where angiogenesis is desired such as peripheral vascular disease, hypertension, inflammatory vasculitides, Reynaud's disease and Reynaud's phenomenon, aneurysms, arterial restenosis, thrombophlebitis, lymphangitis, lymphedema, wound healing and tissue repair, ischemia reperfusion injury, angina, myocardial infarctions such as acute myocardial infarctions, chronic heart conditions, heart failure such as congestive heart failure, and osteoporosis.

If, however, the molecule inhibits angiogenesis, an antagonist thereof would be used for treatment of those conditions where angiogenesis is desired.

Specific types of diseases are described below, where the PRO polypeptide herein or antagonists thereof may serve as useful for vascular-related drug targeting or as therapeutic targets for the treatment or prevention of the disorders. Atherosclerosis is a disease characterized by accumulation of plaques of intimal thickening in arteries, due to accumulation of lipids, proliferation of smooth muscle cells, and formation of fibrous tissue within the arterial wall. The disease can affect large, medium, and small arteries in any organ. Changes in endothelial and

vascular smooth muscle cell function are known to play an important role in modulating the accumulation and regression of these plaques.

Hypertension is characterized by raised vascular pressure in the systemic arterial, pulmonary arterial, or portal venous systems. Elevated pressure may result from or result in impaired endothelial function and/or vascular disease.

Inflammatory vasculitides include giant cell arteritis, Takayasu's arteritis, polyarteritis nodosa (including the microangiopathic form), Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, and a variety of infectious-related vascular disorders (including Henoch-Schonlein purpura). Altered endothelial cell function has been shown to be important in these diseases.

Reynaud's disease and Reynaud's phenomenon are characterized by intermittent abnormal impairment of the circulation through the extremities on exposure to cold. Altered endothelial cell function has been shown to be important in this disease.

Aneurysms are saccular or fusiform dilatations of the arterial or venous tree that are associated with altered endothelial cell and/or vascular smooth muscle cells.

Arterial restenosis (restenosis of the arterial wall) may occur following angioplasty as a result of alteration in the function and proliferation of endothelial and vascular smooth muscle cells.

Thrombophlebitis and lymphangitis are inflammatory disorders of veins and lymphatics, respectively, that may result from, and/or in, altered endothelial cell function. Similarly, lymphedema is a condition involving impaired lymphatic vessels resulting from endothelial cell function.

The family of benign and malignant vascular tumors are characterized by abnormal proliferation and growth of cellular elements of the vascular system. For example, lymphangiomas are benign tumors of the lymphatic system that are congenital, often cystic, malformations of the lymphatics that usually occur in newborns. Cystic tumors tend to grow into the adjacent tissue. Cystic tumors usually occur in the cervical and axillary region. They can also occur in the soft tissue of the extremities. The main symptoms are dilated, sometimes reticular, structured lymphatics and lymphocysts surrounded by connective tissue. Lymphangiomas are assumed to be caused by improperly connected embryonic lymphatics or their deficiency. The result is impaired local lymph drainage. Griener *et al.*, *Lymphology*, 4: 140-144 (1971).

Another use for the PRO polypeptides herein or antagonists thereto is in the prevention of tumor angiogenesis, which involves vascularization of a tumor to enable it to grow and/or metastasize. This process is dependent on the growth of new blood vessels. Examples of neoplasms and related conditions that involve tumor angiogenesis include breast carcinomas, lung carcinomas, gastric carcinomas, esophageal carcinomas, colorectal carcinomas, liver carcinomas, ovarian carcinomas, thecomas, arrhenoblastomas, cervical carcinomas, endometrial carcinoma, endometrial hyperplasia, endometriosis, fibrosarcomas, choriocarcinoma, head and neck cancer, nasopharyngeal carcinoma, laryngeal carcinomas, hepatoblastoma, Kaposi's sarcoma, melanoma, skin carcinomas, hemangioma, cavernous hemangioma, hemangioblastoma, pancreas carcinomas, retinoblastoma, astrocytoma, glioblastoma, Schwannoma, oligodendroglioma, medulloblastoma, neuroblastomas, rhabdomyosarcoma, osteogenic sarcoma, leiomyosarcomas, urinary tract carcinomas, thyroid carcinomas, Wilm's tumor, renal cell carcinoma, prostate carcinoma, abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain

tumors), and Meigs' syndrome.

Age-related macular degeneration (AMD) is a leading cause of severe visual loss in the elderly population. The exudative form of AMD is characterized by choroidal neovascularization and retinal pigment epithelial cell detachment. Because choroidal neovascularization is associated with a dramatic worsening in prognosis, the PRO polypeptide or antagonist thereto is expected to be useful in reducing the severity of AMD.

Healing of trauma such as wound healing and tissue repair is also a targeted use for the PRO polypeptides herein or their antagonists. Formation and regression of new blood vessels is essential for tissue healing and repair. This category includes bone, cartilage, tendon, ligament, and/or nerve tissue growth or regeneration, as well as wound healing and tissue repair and replacement, and in the treatment of burns, incisions, and ulcers. A PRO polypeptide or antagonist thereof that induces cartilage and/or bone growth in circumstances where bone is not normally formed has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a PRO polypeptide or antagonist thereof may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. *De novo* bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma-induced, or oncologic, resection-induced craniofacial defects, and also is useful in cosmetic plastic surgery.

PRO polypeptides or antagonists thereto may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a PRO polypeptide or antagonist thereto may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, or endothelium), muscle (smooth, skeletal, or cardiac), and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate.

A PRO polypeptide herein or antagonist thereto may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage. Also, the PRO polypeptide or antagonist thereto may be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells, or for inhibiting the growth of tissues described above.

A PRO polypeptide or antagonist thereto may also be used in the treatment of periodontal diseases and in other tooth-repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells, or induce differentiation of progenitors of bone-forming cells. A PRO polypeptide herein or an antagonist thereto may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes, since blood vessels play an important role in the regulation of bone turnover and growth.

Another category of tissue regeneration activity that may be attributable to the PRO polypeptide herein or antagonist thereto is tendon/ligament formation. A protein that induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed has application in the healing of tendon or

ligament tears, deformities, and other tendon or ligament defects in humans and other animals. Such a preparation may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. *De novo* tendon/ligament-like tissue formation induced by a composition of the PRO polypeptide herein or antagonist thereto contributes to the repair of congenital, trauma-induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions herein may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions herein may also be useful in the treatment of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The PRO polypeptide or its antagonist may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.*, for the treatment of central and peripheral nervous system disease and neuropathies, as well as mechanical and traumatic disorders, that involve degeneration, death, or trauma to neural cells or nerve tissue. More specifically, a PRO polypeptide or its antagonist may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions that may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma, and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a PRO polypeptide herein or antagonist thereto.

Ischemia-reperfusion injury is another indication. Endothelial cell dysfunction may be important in both the initiation of, and in regulation of the sequelae of events that occur following ischemia-reperfusion injury.

Rheumatoid arthritis is a further indication. Blood vessel growth and targeting of inflammatory cells through the vasculature is an important component in the pathogenesis of rheumatoid and sero-negative forms of arthritis.

A PRO polypeptide or its antagonist may also be administered prophylactically to patients with cardiac hypertrophy, to prevent the progression of the condition, and avoid sudden death, including death of asymptomatic patients. Such preventative therapy is particularly warranted in the case of patients diagnosed with massive left ventricular cardiac hypertrophy (a maximal wall thickness of 35 mm or more in adults, or a comparable value in children), or in instances when the hemodynamic burden on the heart is particularly strong.

A PRO polypeptide or its antagonist may also be useful in the management of atrial fibrillation, which develops in a substantial portion of patients diagnosed with hypertrophic cardiomyopathy.

Further indications include angina, myocardial infarctions such as acute myocardial infarctions, and heart failure such as congestive heart failure. Additional non-neoplastic conditions include psoriasis, diabetic and other proliferative retinopathies including retinopathy of prematurity, retrolental fibroplasia, neovascular glaucoma, thyroid hyperplasias (including Grave's disease), corneal and other tissue transplantation, chronic inflammation, lung inflammation, nephrotic syndrome, preeclampsia, ascites, pericardial effusion (such as that associated with

pericarditis), and pleural effusion.

In view of the above, the PRO polypeptides or agonists or antagonists thereof described herein, which are shown to alter or impact endothelial cell function, proliferation, and/or form, are likely to play an important role in the etiology and pathogenesis of many or all of the disorders noted above, and as such can serve as therapeutic targets to augment or inhibit these processes or for vascular-related drug targeting in these disorders.

xi. Administration Protocols, Schedules, Doses, and Formulations

The molecules herein and agonists and antagonists thereto are pharmaceutically useful as a prophylactic and therapeutic agent for various disorders and diseases as set forth above.

Therapeutic compositions of the PRO polypeptides or agonists or antagonists are prepared for storage by mixing the desired molecule having the appropriate degree of purity with optional pharmaceutically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, 16th edition, Osol, A. ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEENTM, PLURONICSTM or polyethylene glycol (PEG).

Additional examples of such carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts, or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, and polyethylene glycol. Carriers for topical or gel-based forms of antagonist include polysaccharides such as sodium carboxymethylcellulose or methylcellulose, polyvinylpyrrolidone, polyacrylates, polyoxyethylene-polyoxypropylene-block polymers, polyethylene glycol, and wood wax alcohols. For all administrations, conventional depot forms are suitably used. Such forms include, for example, microcapsules, nano-capsules, liposomes, plasters, inhalation forms, nose sprays, sublingual tablets, and sustained-release preparations. The PRO polypeptides or agonists or antagonists will typically be formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml.

Another formulation comprises incorporating a PRO polypeptide or antagonist thereof into formed articles. Such articles can be used in modulating endothelial cell growth and angiogenesis. In addition, tumor invasion and metastasis may be modulated with these articles.

PRO polypeptides or antagonists to be used for *in vivo* administration must be sterile. This is readily

accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. PRO polypeptides ordinarily will be stored in lyophilized form or in solution if administered systemically. If in lyophilized form, the PRO polypeptide or antagonist thereto is typically formulated in combination with other ingredients for reconstitution with an appropriate diluent at the time for use. An example of a liquid formulation of a PRO polypeptide or antagonist is a sterile, clear, colorless unpreserved solution filled in a single-dose vial for subcutaneous injection. Preserved pharmaceutical compositions suitable for repeated use may contain, for example, depending mainly on the indication and type of polypeptide:

- a) PRO polypeptide or agonist or antagonist thereto;
- b) a buffer capable of maintaining the pH in a range of maximum stability of the polypeptide or other molecule in solution, preferably about 4-8;
- c) a detergent/surfactant primarily to stabilize the polypeptide or molecule against agitation-induced aggregation;
- d) an isotonifier;
- e) a preservative selected from the group of phenol, benzyl alcohol and a benzethonium halide, *e.g.*, chloride;
- f) water.

If the detergent employed is non-ionic, it may, for example, be polysorbates (*e.g.*, POLYSORBATE™ (TWEEN™) 20, 80, etc.) or poloxamers (*e.g.*, POLOXAMER™ 188). The use of non-ionic surfactants permits the formulation to be exposed to shear surface stresses without causing denaturation of the polypeptide. Further, such surfactant-containing formulations may be employed in aerosol devices such as those used in a pulmonary dosing, and needleless jet injector guns (*see, e.g.*, EP 257,956).

An isotonifier may be present to ensure isotonicity of a liquid composition of the PRO polypeptide or antagonist thereto, and includes polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol, and mannitol. These sugar alcohols can be used alone or in combination. Alternatively, sodium chloride or other appropriate inorganic salts may be used to render the solutions isotonic.

The buffer may, for example, be an acetate, citrate, succinate, or phosphate buffer depending on the pH desired. The pH of one type of liquid formulation of this invention is buffered in the range of about 4 to 8, preferably about physiological pH.

The preservatives phenol, benzyl alcohol and benzethonium halides, *e.g.*, chloride, are known antimicrobial agents that may be employed.

Therapeutic PRO polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle. The formulations are preferably administered as repeated intravenous (*i.v.*), subcutaneous (*s.c.*), or intramuscular (*i.m.*) injections, or as aerosol formulations suitable for intranasal or intrapulmonary delivery (for intrapulmonary delivery *see, e.g.*, EP 257,956).

PRO polypeptides can also be administered in the form of sustained-released preparations. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the

protein, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (e.g., poly(2-hydroxyethyl-methacrylate) as described by Langer *et al.*, J. Biomed. Mater. Res., 15: 167-277 (1981) and Langer, Chem. Tech., 12: 98-105 (1982) or poly(vinylalcohol)), polylactides (U.S. Patent No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, Biopolymers, 22: 547-556 (1983)), non-degradable ethylene-vinyl acetate (Langer *et al.*, *supra*), degradable lactic acid-glycolic acid copolymers such as the Lupron Depot™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated proteins remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for protein stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Sustained-release PRO polypeptide compositions also include liposomally entrapped PRO polypeptides. Liposomes containing the PRO polypeptide are prepared by methods known *per se*: DE 3,218,121; Epstein *et al.*, Proc. Natl. Acad. Sci. USA, 82: 3688-3692 (1985); Hwang *et al.*, Proc. Natl. Acad. Sci. USA, 77: 4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese patent application 83-118008; U.S. Patent Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. % cholesterol, the selected proportion being adjusted for the optimal therapy.

The therapeutically effective dose of a PRO polypeptide or antagonist thereto will, of course, vary depending on such factors as the pathological condition to be treated (including prevention), the method of administration, the type of compound being used for treatment, any co-therapy involved, the patient's age, weight, general medical condition, medical history, etc., and its determination is well within the skill of a practicing physician. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the maximal therapeutic effect. If the PRO polypeptide has a narrow host range, for the treatment of human patients formulations comprising human PRO polypeptide, more preferably native-sequence human PRO polypeptide, are preferred. The clinician will administer the PRO polypeptide until a dosage is reached that achieves the desired effect for treatment of the condition in question. For example, if the objective is the treatment of CHF, the amount would be one that inhibits the progressive cardiac hypertrophy associated with this condition. The progress of this therapy is easily monitored by echo cardiography. Similarly, in patients with hypertrophic cardiomyopathy, the PRO polypeptide can be administered on an empirical basis.

With the above guidelines, the effective dose generally is within the range of from about 0.001 to about 1.0 mg/kg, more preferably about 0.01-1.0 mg/kg, most preferably about 0.01-0.1 mg/kg.

For non-oral use in treating human adult hypertension, it is advantageous to administer the PRO polypeptide in the form of an injection at about 0.01 to 50 mg, preferably about 0.05 to 20 mg, most preferably 1 to 20 mg, per

kg body weight, 1 to 3 times daily by intravenous injection. For oral administration, a molecule based on the PRO polypeptide is preferably administered at about 5 mg to 1 g, preferably about 10 to 100 mg. per kg body weight, 1 to 3 times daily. It should be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less than 0.5 ng/mg protein. Moreover, for human administration, the formulations preferably meet

5 sterility, pyrogenicity, general safety, and purity as required by FDA Office and Biologics standards.

The dosage regimen of a pharmaceutical composition containing the PRO polypeptide to be used in tissue regeneration will be determined by the attending physician considering various factors that modify the action of the polypeptides, *e.g.*, amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (*e.g.*, bone), the patient's age, sex, and diet, the severity of any

10 infection, time of administration, and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF-I, to the final composition may also affect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations, and tetracycline labeling.

The route of PRO polypeptide or antagonist or agonist administration is in accord with known methods, *e.g.*, by injection or infusion by intravenous, intramuscular, intracerebral, intraperitoneal, intracerebrospinal, subcutaneous, intraocular, intraarticular, intrasynovial, intrathecal, oral, topical, or inhalation routes, or by sustained-release systems as noted below. The PRO polypeptide or antagonists thereof also are suitably administered by intratumoral, peritumoral, intralesional, or perilesional routes, to exert local as well as systemic

15 therapeutic effects. The intraperitoneal route is expected to be particularly useful, for example, in the treatment of ovarian tumors.

If a peptide or small molecule is employed as an antagonist or agonist, it is preferably administered orally or non-orally in the form of a liquid or solid to mammals.

Examples of pharmacologically acceptable salts of molecules that form salts and are useful hereunder include

25 alkali metal salts (*e.g.*, sodium salt, potassium salt), alkaline earth metal salts (*e.g.*, calcium salt, magnesium salt), ammonium salts, organic base salts (*e.g.*, pyridine salt, triethylamine salt), inorganic acid salts (*e.g.*, hydrochloride, sulfate, nitrate), and salts of organic acid (*e.g.*, acetate, oxalate, p-toluenesulfonate).

For compositions herein that are useful for bone, cartilage, tendon, or ligament regeneration, the therapeutic method includes administering the composition topically, systemically, or locally as an implant or device. When

30 administered, the therapeutic composition for use is in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage, or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Preferably, for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone

35 and cartilage and preferably capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance, and interface properties. The particular application of the compositions will define the appropriate

formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalcium phosphate, hydroxyapatite, polylactic acid, polyglycolic acid, and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above-mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalcium phosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

One specific embodiment is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the polypeptide compositions from disassociating from the matrix.

One suitable family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, and carboxymethylcellulose, one preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer, and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt%, preferably 1-10 wt%, based on total formulation weight, which represents the amount necessary to prevent desorption of the polypeptide (or its antagonist) from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the polypeptide (or its antagonist) the opportunity to assist the osteogenic activity of the progenitor cells.

xii. Combination Therapies

The effectiveness of the PRO polypeptide or an agonist or antagonist thereof in preventing or treating the disorder in question may be improved by administering the active agent serially or in combination with another agent that is effective for those purposes, either in the same composition or as separate compositions.

For example, for treatment of cardiac hypertrophy, PRO polypeptide therapy can be combined with the administration of inhibitors of known cardiac myocyte hypertrophy factors, *e.g.*, inhibitors of α -adrenergic agonists such as phenylephrine; endothelin-1 inhibitors such as BOSENTANTM and MOXONODINTM; inhibitors to CT-1 (U.S. Pat. No. 5,679,545); inhibitors to LIF; ACE inhibitors; des-aspartate-angiotensin I inhibitors (U.S. Pat. No. 5,773,415), and angiotensin II inhibitors.

For treatment of cardiac hypertrophy associated with hypertension, the PRO polypeptide can be administered in combination with β -adrenergic receptor blocking agents, *e.g.*, propranolol, timolol, tertalolol, carteolol, nadolol, betaxolol, penbutolol, acetobutolol, atenolol, metoprolol, or carvedilol; ACE inhibitors, *e.g.*, quinapril, captopril, enalapril, ramipril, benazepril, fosinopril, or lisinopril; diuretics, *e.g.*, chlorothiazide, hydrochlorothiazide, hydroflumethazide, methylchlorothiazide, benzthiazide, dichlorphenamide, acetazolamide, or indapamide; and/or calcium channel blockers, *e.g.*, diltiazem, nifedipine, verapamil, or nifedipine. Pharmaceutical compositions

comprising the therapeutic agents identified herein by their generic names are commercially available, and are to be administered following the manufacturers' instructions for dosage, administration, adverse effects, contraindications, etc. See, e.g., Physicians' Desk Reference (Medical Economics Data Production Co.: Montvale, N.J., 1997), 51th Edition.

- 5 Preferred candidates for combination therapy in the treatment of hypertrophic cardiomyopathy are β -adrenergic-blocking drugs (e.g., propranolol, timolol, tertalolol, carteolol, nadolol, betaxolol, penbutolol, acetobutolol, atenolol, metoprolol, or carvedilol), verapamil, diltiazem, or diltiazem. Treatment of hypertrophy associated with high blood pressure may require the use of antihypertensive drug therapy, using calcium channel blockers, e.g., diltiazem, nifedipine, verapamil, or nicardipine; β -adrenergic blocking agents; diuretics, e.g.,
10 chlorothiazide, hydrochlorothiazide, hydroflumethazide, methylchlorothiazide, benzthiazide, dichlorphenamide, acetazolamide, or indapamide; and/or ACE-inhibitors, e.g., quinapril, captopril, enalapril, ramipril, benazepril, fosinopril, or lisinopril.

- For other indications, PRO polypeptides or their antagonists may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various
15 growth factors such as EGF, PDGF, TGF- α or TGF- β , IGF, FGF, and CTGF.

In addition, PRO polypeptides or their antagonists used to treat cancer may be combined with cytotoxic, chemotherapeutic, or growth-inhibitory agents as identified above. Also, for cancer treatment, the PRO polypeptide or antagonist thereof is suitably administered serially or in combination with radiological treatments, whether involving irradiation or administration of radioactive substances.

- 20 The effective amounts of the therapeutic agents administered in combination with the PRO polypeptide or antagonist thereof will be at the physician's or veterinarian's discretion. Dosage administration and adjustment is done to achieve maximal management of the conditions to be treated. For example, for treating hypertension, these amounts ideally take into account use of diuretics or digitalis, and conditions such as hyper- or hypotension, renal impairment, etc. The dose will additionally depend on such factors as the type of the therapeutic agent to be used
25 and the specific patient being treated. Typically, the amount employed will be the same dose as that used, if the given therapeutic agent is administered without the PRO polypeptide.

xiii. Articles of Manufacture

- An article of manufacture such as a kit containing the PRO polypeptide or agonists or antagonists thereof useful for the diagnosis or treatment of the disorders described above comprises at least a container and a label.
30 Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition that is effective for diagnosing or treating the condition and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is the PRO polypeptide or an agonist or antagonist thereto. The label on, or associated with, the
35 container indicates that the composition is used for diagnosing or treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution, and dextrose solution. It may further include other materials desirable

from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. The article of manufacture may also comprise a second or third container with another active agent as described above.

E. Antibodies

5 Some of the most promising drug candidates according to the present invention are antibodies and antibody fragments that may inhibit the production or the gene product of the genes identified herein and/or reduce the activity of the gene products.

i. Polyclonal Antibodies

10 Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include, but are not limited to, keyhole limpet hemocyanin, 15 serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants that may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A or synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

ii. Monoclonal Antibodies

20 The anti-PRO antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

25 The immunizing agent will typically include the PRO polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. Goding, Monoclonal Antibodies: Principles and Practice (New York: Academic Press, 1986), pp. 59-103. Immortalized cell lines are 30 usually transformed mammalian cells, particularly myeloma cells of rodent, bovine, and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine 35 ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high-level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies. Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, Monoclonal Antibody Production Techniques and Applications (Marcel Dekker, Inc.: New York, 1987) pp. 51-63.

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the PRO polypeptide. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, *Anal. Biochem.*, 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods. Goding, *supra*. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison *et al.*, *supra*) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy-chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using routine techniques known in the art.

iii. Human and Humanized Antibodies

The anti-PRO antibodies may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin, and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody preferably also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Jones *et al.*, Nature, **321**: 522-525 (1986); Riechmann *et al.*, Nature, **332**: 323-329 (1988); Presta, Curr. Op. Struct. Biol., **2**: 593-596 (1992).

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, Nature, **321**: 522-525 (1986); Riechmann *et al.*, Nature, **332**: 323-327 (1988); Verhoeven *et al.*, Science, **239**: 1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries. Hoogenboom and Winter, J. Mol. Biol., **227**: 381 (1991); Marks *et al.*, J. Mol. Biol., **222**: 581 (1991). The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies. Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner *et al.*, J. Immunol., **147**(1): 86-95 (1991). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed that closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016, and in the following scientific publications: Marks *et al.*, Bio/Technology, **10**: 779-783 (1992); Lonberg

et al., Nature, **368**: 856-859 (1994); Morrison, Nature, **368**: 812-813 (1994); Fishwild *et al.*, Nature Biotechnology, **14**: 845-851 (1996); Neuberger, Nature Biotechnology, **14**: 826 (1996); Lonberg and Huszar, Intern. Rev. Immunol., **13**: 65-93 (1995).

iv. Bispecific Antibodies

5 Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO polypeptide, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

10 Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities. Milstein and Cuello, Nature, **305**: 537-539 (1983). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures
15 are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, EMBO J., **10**: 3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant-domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of
20 the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies, *see*, for example, Suresh *et al.*, Methods in Enzymology, **121**: 210 (1986).

v. Heteroconjugate Antibodies

25 Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune-system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection. WO 91/00360; WO 92/200373; EP 03089. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a
30 thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptoputyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

vi. Effector Function Engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*, the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) may be introduced into
35 the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus

generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See, Caron *et al.*, J. Exp. Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.*, Cancer Research, 53: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See, Stevenson *et al.*, Anti-Cancer Drug Design, 3: 219-230 (1989).

vii. Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, saponaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi , ^{131}I , ^{131}In , ^{90}Y , and ^{186}Re .

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See, WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.*, avidin) that is conjugated to a cytotoxic agent (*e.g.*, a radionucleotide).

viii. Immunoliposomes

The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang *et al.*, Proc. Natl. Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid

composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al.*, J. Biol. Chem., 257: 286-288 (1982) via a disulfide-interchange reaction. A chemotherapeutic agent (such as
5 Doxorubicin) is optionally contained within the liposome. See, Gabizon *et al.*, J. National Cancer Inst., 81(19): 1484 (1989).

ix. Pharmaceutical Compositions of Antibodies

Antibodies specifically binding a PRO polypeptide identified herein, as well as other molecules identified by the screening assays disclosed hereinbefore, can be administered for the treatment of various disorders as noted
10 above and below in the form of pharmaceutical compositions.

If the PRO polypeptide is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody,
15 peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. See, e.g., Marasco *et al.*, Proc. Natl. Acad. Sci. USA, 90: 7889-7893 (1993).

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other.
20 Alternatively, or in addition, the composition may comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-
25 (methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, *supra*.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

30 Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as
35 ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels

release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C. resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

x. Methods of Treatment using the Antibody

It is contemplated that the antibodies to a PRO polypeptide may be used to treat various cardiovascular, endothelial, and angiogenic conditions as noted above.

The antibodies are administered to a mammal, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous administration of the antibody is preferred.

Other therapeutic regimens may be combined with the administration of the antibodies of the instant invention as noted above. For example, if the antibodies are to treat cancer, the patient to be treated with such antibodies may also receive radiation therapy. Alternatively, or in addition, a chemotherapeutic agent may be administered to the patient. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service, Ed., M.C. Perry (Williams & Wilkins: Baltimore, MD, 1992). The chemotherapeutic agent may precede, or follow administration of the antibody, or may be given simultaneously therewith. The antibody may be combined with an anti-estrogen compound such as tamoxifen or EVISTA™ or an anti-progesterone such as onapristone (see, EP 616812) in dosages known for such molecules.

If the antibodies are used for treating cancer, it may be desirable also to administer antibodies against other tumor-associated antigens, such as antibodies that bind to one or more of the ErbB2, EGFR, ErbB3, ErbB4, or VEGF receptor(s). These also include the agents set forth above. Also, the antibody is suitably administered serially or in combination with radiological treatments, whether involving irradiation or administration of radioactive substances. Alternatively, or in addition, two or more antibodies binding the same or two or more different antigens disclosed herein may be co-administered to the patient. Sometimes, it may be beneficial also to administer one or more cytokines to the patient. In a preferred embodiment, the antibodies herein are co-administered with a growth-inhibitory agent. For example, the growth-inhibitory agent may be administered first, followed by an antibody of the present invention. However, simultaneous administration or administration of the antibody of the present invention first is also contemplated. Suitable dosages for the growth-inhibitory agent are those presently used and may be lowered due to the combined action (synergy) of the growth-inhibitory agent and the antibody herein.

In one embodiment, vascularization of tumors is attacked in combination therapy. The anti-PRO polypeptide antibody and another antibody (e.g., anti-VEGF) are administered to tumor-bearing patients at therapeutically effective doses as determined, for example, by observing necrosis of the tumor or its metastatic foci, if any. This therapy is continued until such time as no further beneficial effect is observed or clinical examination shows no trace of the tumor or any metastatic foci. Then TNF is administered, alone or in combination with an auxiliary agent such as alpha-, beta-, or gamma-interferon, anti-HER2 antibody, heregulin, anti-heregulin antibody, D-factor, interleukin-1 (IL-1), interleukin-2 (IL-2), granulocyte-macrophage colony stimulating factor (GM-CSF), or agents that promote microvascular coagulation in tumors, such as anti-protein C antibody, anti-protein S antibody, or C4b binding protein (*see*, WO 91/01753, published 21 February 1991), or heat or radiation.

Since the auxiliary agents will vary in their effectiveness, it is desirable to compare their impact on the tumor by matrix screening in conventional fashion. The administration of anti-PRO polypeptide antibody and TNF is repeated until the desired clinical effect is achieved. Alternatively, the anti-PRO polypeptide antibody is administered together with TNF and, optionally, auxiliary agent(s). In instances where solid tumors are found in the limbs or in other locations susceptible to isolation from the general circulation, the therapeutic agents described herein are administered to the isolated tumor or organ. In other embodiments, a FGF or PDGF antagonist, such as an anti-FGF or an anti-PDGF neutralizing antibody, is administered to the patient in conjunction with the anti-PRO polypeptide antibody. Treatment with anti-PRO polypeptide antibodies preferably may be suspended during periods of wound healing or desirable neovascularization.

For the prevention or treatment of cardiovascular, endothelial, and angiogenic disorder, the appropriate dosage of an antibody herein will depend on the type of disorder to be treated, as defined above, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments.

For example, depending on the type and severity of the disorder, about 1 $\mu\text{g/kg}$ to 50 mg/kg (e.g., 0.1-20 mg/kg) of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily or weekly dosage might range from about 1 $\mu\text{g/kg}$ to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated or sustained until a desired suppression of disorder symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays, including, for example, radiographic tumor imaging.

xi. Articles of Manufacture with Antibodies

An article of manufacture containing a container with the antibody and a label is also provided. Such articles are described above, wherein the active agent is an anti-PRO antibody.

xii. Diagnosis and Prognosis of Tumors using Antibodies

If the indication for which the antibodies are used is cancer, while cell-surface proteins, such as growth receptors over expressed in certain tumors, are excellent targets for drug candidates or tumor (e.g., cancer)

treatment, the same proteins along with PRO polypeptides find additional use in the diagnosis and prognosis of tumors. For example, antibodies directed against the PRO polypeptides may be used as tumor diagnostics or prognostics.

For example, antibodies, including antibody fragments, can be used qualitatively or quantitatively to detect the expression of genes including the gene encoding the PRO polypeptide. The antibody preferably is equipped with a detectable, e.g., fluorescent label, and binding can be monitored by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. Such binding assays are performed essentially as described above.

In situ detection of antibody binding to the marker gene products can be performed, for example, by immunofluorescence or immunoelectron microscopy. For this purpose, a histological specimen is removed from the patient, and a labeled antibody is applied to it, preferably by overlaying the antibody on a biological sample. This procedure also allows for determining the distribution of the marker gene product in the tissue examined. It will be apparent to those skilled in the art that a wide variety of histological methods are readily available for *in situ* detection.

The following Examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

The disclosures of all patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

EXAMPLES

Commercially available reagents referred to in the Examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following Examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas, VA. Unless otherwise noted, the present invention uses standard procedures of recombinant DNA technology, such as those described hereinabove and in the following textbooks: Sambrook *et al.*, *supra*; Ausubel *et al.*, Current Protocols in Molecular Biology (Green Publishing Associates and Wiley Interscience, N.Y., 1989); Innis *et al.*, PCR Protocols: A Guide to Methods and Applications (Academic Press, Inc.: N.Y., 1990); Harlow *et al.*, Antibodies: A Laboratory Manual (Cold Spring Harbor Press: Cold Spring Harbor, 1988); Gait, Oligonucleotide Synthesis (IRL Press: Oxford, 1984); Freshney, Animal Cell Culture, 1987; Coligan *et al.*, Current Protocols in Immunology, 1991.

EXAMPLE 1

Extracellular Domain Homology Screening to Identify Novel Polypeptides and cDNA Encoding Therefor

The extracellular domain (ECD) sequences (including the secretion signal sequence, if any) from about 950 known secreted proteins from the Swiss-Prot public database were used to search EST databases. The EST databases included public databases (e.g., GenBank), and proprietary databases (e.g., LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST-2 [Altschul *et al.*, Methods in Enzymology, 266:460-480 (1996)] as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. Those comparisons with a BLAST score of 70 (or in some cases, 90)

or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

Using this extracellular domain homology screen, consensus DNA sequences were assembled relative to other identified EST sequences using phrap. In addition, the consensus DNA sequences obtained were often (but not always) extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above.

Based upon the consensus sequences obtained as described above, oligonucleotides were then synthesized and used to identify by PCR a cDNA library that contained the sequence of interest and for use as probes to isolate a clone of the full-length coding sequence for a PRO polypeptide. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5 kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al.*, Current Protocols in Molecular Biology, *supra*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; *see*, Holmes *et al.*, Science, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

EXAMPLE 2

Isolation of cDNA Clones Using Signal Algorithm Analysis

Various polypeptide-encoding nucleic acid sequences were identified by applying a proprietary signal sequence finding algorithm developed by Genentech, Inc., (South San Francisco, CA) upon ESTs as well as clustered and assembled EST fragments from public (*e.g.*, GenBank) and/or private (LIFESEQ®, Incyte Pharmaceuticals, Inc., Palo Alto, CA) databases. The signal sequence algorithm computes a secretion signal score based on the character of the DNA nucleotides surrounding the first and optionally the second methionine codon(s) (ATG) at the 5'-end of the sequence or sequence fragment under consideration. The nucleotides following the first ATG must code for at least 35 unambiguous amino acids without any stop codons. If the first ATG has the required amino acids, the second is not examined. If neither meets the requirement, the candidate sequence is not scored. In order to determine whether the EST sequence contains an authentic signal sequence, the DNA and corresponding amino acid sequences surrounding the ATG codon are scored using a set of seven sensors (evaluation parameters) known to be associated with secretion signals. Use of this algorithm resulted in the identification of numerous polypeptide-encoding nucleic acid sequences.

EXAMPLE 3

Isolation of cDNA Clones Encoding Human PRO175

A cDNA clone (DNA19355-1150-P1) encoding a native human PRO175 polypeptide was identified using a yeast screen, in a human umbilical vein endothelium cell cDNA library that preferentially represents the 5' ends of the primary cDNA clones.

A full length clone was identified that contained a single open reading frame with an apparent translational initiation site at nucleotide positions 21-23, and a stop signal at nucleotide positions 552-554 (Figure 1, SEQ ID NO:1). The predicted polypeptide precursor is 177 amino acids long, and has a calculated molecular weight of approximately 20,308 daltons.

Analysis of the full-length PRO175 sequence shown in Figure 2 (SEQ ID NO:2) evidenced the presence of a variety of important polypeptide domains as shown in Figure 2, wherein the locations given for those important polypeptide domains are approximate as described above. Analysis of the full-length PRO175 sequence (Figure 2; SEQ ID NO:2) evidences a type II transmembrane protein typology and the presence of the following: a signal peptide from about amino acid 1 to about amino acid 25; a transmembrane domain from about amino acid 26 to about amino acid 51; an extracellular domain from about amino acid 52 to about amino acid 177; potential N-linked glycosylation sites from about amino acid 129 to about amino acid 133 and from about amino acid 161 to about amino acid 165; and an N-myristoylation site from about amino acid 18 to about amino acid 24. Clone DNA19355-1150-P1 has been deposited with ATCC on November 18, 1997 and is assigned ATCC deposit no. 209466.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using the ALIGN-2 sequence alignment analysis of the full-length sequence shown in Figure 2 (SEQ ID NO:2), evidenced significant identity between the PRO175 amino acid sequence and several members of the TNF cytokine family, and particularly, to human Apo-2L (19.8%), Fas/Apo1-ligand (19.0%), TNF-alpha (20.6%) and Lymphotoxin- α (17.5%). Most of the amino acid sequence identity is found in the regions corresponding to the beta-strands in the crystal structure of TNF- α [Banner *et al.*, *Cell*, **73**:431-435 (1993); Eck *et al.*, *J. Biol. Chem.*, **264**:17595-605 (1989); Lewit-Bentley *et al.*, *J. Mol. Biol.*, **199**:389-92 (1988)]. The sequence of strand C is especially conserved in all members of the family. The sequence between the putative transmembrane domain and the first beta-strand of the DNA19355-1150-P1 polypeptide is relatively short, including 5 residues, as compared to about 30 to about 80 residues in TNF- α , CD95L or Apo-2 ligand.

EXAMPLE 4

Isolation of cDNA Clones Encoding Human PRO200

An expressed sequence tag (EST) DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) was searched and Incyte EST clone "INC1302516" was identified that had homology to VEGF. Probes based on the Incyte EST clone "INC1302516" were used to screen a cDNA library derived from the human glioma cell line G61. In particular, Incyte clone "INC1302516" was used to generate the following four probes:

5'-ACTTCTCAGTGTCATAAGGG-3'	(SEQ ID NO:35)
5'-GAACTAAAGAGAACCGATACCATTTTCTGGCCAGGTTGTC-3'	(SEQ ID NO:36)
5'-CACACAGCGTTTAACCAGG-3'	(SEQ ID NO:37)

5'-ACAACAGGCACAGTTCAC-3'

(SEQ ID NO:38)

The cDNA libraries used to isolate the cDNA clones encoding human PRO200 were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; *see*, Holmes *et al.*, Science, 253:1278-1280 (1991)) in the unique XhoI and NotI.

A cDNA clone was identified and sequenced in entirety. The entire nucleotide sequence of DNA29101-1122 is shown in Figure 3 (SEQ ID NO:3). Clone DNA29101-1122 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 285-287, and a stop codon at nucleotide positions 1320-1322 (Figure 3; SEQ ID NO:3). The predicted polypeptide precursor is 345 amino acids long. The full-length PRO200 protein is shown in Figure 4 (SEQ ID NO:4).

Analysis of the full-length PRO200 sequence shown in Figure 4 (SEQ ID NO:4) evidences the presence of important polypeptide domains as shown in Figure 4, wherein the location given for those important polypeptide domains are approximate as described above. Analysis of the full-length PRO200 sequence (Figure 4; SEQ ID NO:4) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 14; N-glycosylation sites from about amino acid 25 to about amino acid 29, from about amino acid 55 to about amino acid 59, and from about amino acid 254 to about amino acid 258; N-myristoylation sites from about amino acid 15 to about amino acid 21, from about amino acid 117 to about amino acid 123, from about amino acid 127 to about amino acid 133, from about amino acid 281 to about amino acid 287, from about amino acid 282 to about amino acid 288, and from about amino acid 319 to about amino acid 325; and an amidation site from about amino acid 229 to about amino acid 233.

Clone DNA29101-1122 has been deposited with ATCC on March 5, 1998 and is assigned ATCC deposit no. 209653. It is understood that the deposited clone has the actual correct sequence rather than the representations provided herein.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using the ALIGN-2 sequence alignment analysis of the full-length sequence shown in Figure 4 (SEQ ID NO:4), evidenced some degree of sequence identity between the PRO200 amino acid sequence and the vascular endothelial cell growth factor (VEGF) and the bone morphogenetic protein (VEGF-E polypeptide).

EXAMPLE 5

Isolation of cDNA Clones Encoding Human PRO198

PRO198 was identified by screening the GenBank database using the computer program BLAST (Altschul *et al.*, Methods in Enzymology, 266:460-480 (1996)). The PRO198 sequence was shown to have homology with known EST sequences T57280 and T50719. None of the known EST sequences have been identified as full-length sequences, or described as ligands associated with TIE receptors. Following identification, PRO198 was cloned from a human fetal lung library prepared from mRNA purchased from Clontech, Inc., (Palo Alto, CA), catalog # 6528-1, following the manufacturer's instructions. The library was screened by hybridization with synthetic

oligonucleotide probes.

The oligonucleotide sequences used were as follows:

5'-TGGTTGGCAAAGGCAAGGTGGCTGACGATCCGG-3' (SEQ ID NO:39)

5'-GTGGCCCTTATCTCTCCTGTACAGCTTCCGGATCGTCAGCCAC-3' (SEQ ID NO:40)

5'-TCCATTCCCACCTATGACGCTGACCCA-3' (SEQ ID NO:41)

A cDNA clone was identified and sequenced in entirety. The entire nucleotide sequence of DNA33457-1078 is shown in Figure 5 (SEQ ID NO:5). Clone DNA33457-1078 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 33-35, and a stop codon at nucleotide positions 897-899 (Figure 5; SEQ ID NO:5). The predicted polypeptide precursor is 288 amino acids long. The full-length PRO198 protein is shown in Figure 6 (SEQ ID NO:6).

Analysis of the full-length PRO198 sequence shown in Figure 6 (SEQ ID NO:6) evidences the presence of important polypeptide domains as shown in Figure 6, wherein the locations given for those important polypeptide domains are approximate as described above. Analysis of the full-length PRO198 sequence (Figure 6; SEQ ID NO:6) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 16; an N-glycosylation site from about amino acid 178 to about amino acid 182; a glycosaminoglycan attachment site from about amino acid 272 to about amino acid 276; a tyrosine kinase phosphorylation site from about amino acid 188 to about amino acid 197; N-myristoylation sites from about amino acid 16 to about amino acid 22, from about amino acid 89 to about amino acid 95, from about amino acid 144 to about amino acid 150, and from about amino acid 267 to about amino acid 273; and a fibrinogen beta and gamma chains C-terminal domain signature from about amino acid 242 to about amino acid 255.

Clone DNA33457-1078 has been deposited with ATCC on September 18, 1997 and is assigned ATCC deposit no. 209283. It is understood that the deposited clone has the actual correct sequence rather than the representations provided herein.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using the ALIGN-2 sequence alignment analysis of the full-length sequence shown in Figure 6 (SEQ ID NO:6), evidenced homology between the PRO198 amino acid sequence and ligands associated with TIE receptors. The abbreviation "TIE" is an acronym which stands for "tyrosine kinase containing Ig and EGF homology domains" and was coined to designate a new family of receptor tyrosine kinases.

EXAMPLE 6

Isolation of cDNA Clones Encoding Human PRO364

An expressed sequence tag (EST) DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) was searched and an Incyte EST sequence (Incyte EST No. 3003460) was identified that showed homology to members of the tumor necrosis factor receptor (TNFR) family of polypeptides.

A consensus DNA sequence was then assembled relative to the Incyte 3003460 EST and other EST sequences using repeated cycles of BLAST (Altschul *et al.*, Methods in Enzymology, 266:460-480 (1996)) and "phrap" (Phil Green, University of Washington, Seattle Washington). The consensus sequence is herein designated "<consen01>" and is also herein designated as DNA44825.

Oligonucleotide probes based upon the DNA44825 and "<consen01>" consensus sequences were then synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO364. Forward and reverse PCR primers generally range from 20-30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al.*, Current Protocols in Molecular Biology, *supra*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

Pairs of PCR primers (forward and reverse) were synthesized:

- 10 forward PCR primer (44825.f1):
 5'-CACAGCACGGGGCGATGGG-3' (SEQ ID NO:42)
 forward PCR primer (44825.f2):
 5'-GCTCTGCGTTCTGCTCTG-3' (SEQ ID NO:43)
 forward PCR primer (44825.GITR.f):
 15 5'-GGCACAGCACGGGGCGATGGGCGCGTTT-3' (SEQ ID NO:44)
 reverse PCR primer (44825.r1):
 5'-CTGGTCACTGCCACCTTCCTGCAC-3' (SEQ ID NO:45)
 reverse PCR primer (44825.r2):
 5'-CGCTGACCCAGGCTGAG-3' (SEQ ID NO:46)
 20 reverse PCR primer (44825.GITR.r):
 5'-GAAGGTCCCCGAGGCACAGTCGATACA-3' (SEQ ID NO:47)

Additionally, synthetic oligonucleotide hybridization probes were constructed from the consensus DNA44825 sequence which had the following nucleotide sequences:

- hybridization probe (44825.p1):
 25 5'-GAGGAGTGCTGTTCCGAGTGGGACTGCATGTGTGTCCAGC-3' (SEQ ID NO:48)
 hybridization probe (44825.GITR.p):
 5'-AGCCTGGGTCAGCGCCCCACCGGGGGTCCCGGGTGCGGCC-3' (SEQ ID NO:49)

- In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO364 gene using the probe oligonucleotides and one of the PCR primers.

- RNA for construction of the cDNA libraries was isolated from human small intestine tissue (LIB231). The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; *see*, Holmes *et al.*, Science, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

A cDNA clone was identified and sequenced in entirety. The entire nucleotide sequence of DNA47365-1206

is shown in Figure 7 (SEQ ID NO:7). Clone DNA47365-1206 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 121-123, and a stop codon at nucleotide positions 844-846 (Figure 7; SEQ ID NO:7). The predicted polypeptide precursor is 241 amino acids long and has a predicted molecular weight of approximately 26,000 daltons and an estimated pI of about 6.34. The full-length PRO364 protein is shown in Figure 8 (SEQ ID NO:8).

Analysis of the full-length PRO364 sequence shown in Figure 8 (SEQ ID NO:8) evidences the presence of important polypeptide domains as shown in Figure 8, wherein the locations given for those important polypeptide domains are approximate as described above. Analysis of the full-length PRO364 sequence (Figure 8; SEQ ID NO:8) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 25; a potential transmembrane domain from about amino acid 162 to about amino acid 180; an N-glycosylation site from about amino acid 146 to about amino acid 150; N-myristoylation sites from about amino acid 5 to about amino acid 11, from about amino acid 8 to about amino acid 14, from about amino acid 25 to about amino acid 31, from about amino acid 30 to about amino acid 36, from about amino acid 33 to about amino acid 39, from about amino acid 118 to about amino acid 124, from about amino acid 122 to about amino acid 128, and from about amino acid 156 to about amino acid 162; a prokaryotic membrane lipoprotein lipid attachment site from about amino acid 166 to about amino acid 177; and a leucine zipper pattern from about amino acid 171 to about amino acid 193.

Clone DNA47365-1206 has been deposited with ATCC on November 7, 1997 and is assigned ATCC deposit no. 209436. It is understood that the deposited clone has the actual correct sequence rather than the representations provided herein.

Analysis of the amino acid sequence of the full-length PRO364 polypeptide suggests that portions of it possess homology to members of the tumor necrosis factor receptor family, thereby indicating that PRO364 may be a novel member of the tumor necrosis factor receptor family. The intracellular domain of PRO364 contains a motif (in the region of amino acids 207-214) similar to the minimal domain within CD30 receptor shown to be required for TRAF2 binding and which is also present within TNFR2. There are three apparent extracellular cysteine-rich domains characteristic of the TNFR family (*see*, Naismith and Sprang, Trends Biochem. Sci., 23:74-79 (1998)), of which the third CRD has 3 rather than the more typical 4 or 6 cysteines of the TNFR family. As compared to the mouse GITR the PRO364 amino acid sequence has 8 cysteines in the CRD1 relative to 5 cysteines in CRD1 of mouse GITR, and the presence of one potential N-linked glycosylation site in the ECD as compared to 4 potential N-linked glycosylation sites in mouse GITR.

A detailed review of the putative amino acid sequence of the full-length native PRO364 polypeptide and the nucleotide sequence that encodes it evidences sequence homology with the mouse GITR (mGITR) protein reported by Nocentini *et al.*, Proc. Natl. Acad. Sci. USA, 94:6216-6221 (1997). It is possible, therefore, that PRO364 represents the human counterpart or ortholog to the mouse GITR protein reported by Nocentini *et al.*

EXAMPLE 7

Isolation of cDNA Clones Encoding Human PRO356

An expressed sequence tag (EST) DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) was searched and an EST (#2939340) was identified that had homology to the TIE ligand family. To clone PRO356,

a human uterus mRNA was purchased from Clontech, Inc., (Palo Alto, CA), catalog # 6537-1 was used, following the manufacturer's instructions.

The cDNA libraries used to isolate the cDNA clones encoding human PRO356 were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes *et al.*, Science, 253:1278-1280 (1991)) in the unique XhoI and NotI.

Oligonucleotide probes based upon the above described EST sequence were then synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO356. Forward and reverse PCR primers generally range from 20-30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al.*, Current Protocols in Molecular Biology, *supra*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

The oligonucleotide sequences used were as follows:

5'-TTCAGCACCAAGGACAAGGACAATGACAACT-3' (SEQ ID NO:50)

5'-TGTGCACACTTGTCCAAGCAGTTGTCATTGTC-3' (SEQ ID NO:51)

5'-GTAGTACACTCCATTGAGGTTGG-3' (SEQ ID NO:52)

A cDNA clone was identified and sequenced in entirety. The entire nucleotide sequence of DNA47470-1130-P1 is shown in Figure 9 (SEQ ID NO:9). Clone DNA47470-1130-P1 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 215-217, and a stop codon at nucleotide positions 1253-1255 (Figure 9; SEQ ID NO:9). The predicted polypeptide precursor is 346 amino acids long. The full-length PRO356 protein is shown in Figure 10 (SEQ ID NO:10).

Analysis of the full-length PRO356 sequence shown in Figure 10 (SEQ ID NO:10) evidences the presence of important polypeptide domains as shown in Figure 10, wherein the locations given for those important polypeptide domains are approximate as described above. Analysis of the full-length PRO356 sequence (Figure 10; SEQ ID NO:10) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 26; N-glycosylation sites from about amino acid 58 to about amino acid 62, from about amino acid 253 to about amino acid 257, and from about amino acid 267 to about amino acid 271; a glycosaminoglycan attachment site from about amino acid 167 to about amino acid 171; a cAMP- and cGMP-dependent protein kinase phosphorylation site from about amino acid 176 to about amino acid 180; N-myristoylation sites from about amino acid 168 to about amino acid 174, from about amino acid 196 to about amino acid 202, from about amino acid 241 to about amino acid 247, from about amino acid 252 to about amino acid 258, from about amino acid 256 to about amino acid 262, and from about amino acid 327 to about amino acid 333; and a cell attachment sequence from about amino acid 199 to about amino acid 202.

Clone DNA47470-1130-P1 has been deposited with ATCC on October 28, 1997 and is assigned ATCC

deposit no. 209422. It is understood that the deposited clone has the actual correct sequence rather than the representations provided herein.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using the ALIGN-2 sequence alignment analysis of the full-length sequence shown in Figure 10 (SEQ ID NO:10), shows amino acid sequence identity between the PRO356 amino acid sequence and both TIE-2L1 (32%) and TIE-2L2 (34%). The abbreviation "TIE" is an acronym which stands for "tyrosine kinase containing Ig and EGF homology domains" and was coined to designate a new family of receptor tyrosine kinases.

EXAMPLE 8

Isolation of cDNA Clones Encoding Human PRO535

Use of the signal sequence algorithm described in Example 2 above allowed identification of an EST cluster sequence from the Incyte database, designated herein as ss.clu12694.init. This ss.clu12694.init. EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, Methods in Enzymology, 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases, 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA48352.

In light of an observed sequence homology between the DNA48352 consensus sequence and an EST sequence encompassed within clone no. H86994, from the Merck database, clone H86994 was purchased and the cDNA insert was obtained and sequenced. It was found herein that that insert encoded a full-length protein. The sequence of this cDNA insert is shown in Figure 11 (SEQ ID NO:11) and is herein designated DNA49143-1429.

Clone DNA49143-1429 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 78-80 and ending at the stop codon at nucleotide positions 681-683 (Figure 11). The predicted polypeptide precursor is 201 amino acids long (Figure 12, SEQ ID NO:12). The full-length PRO535 protein shown in Figure 12 has an estimated molecular weight of about 22,180 daltons and a pI of about 9.68.

Analysis of the full-length PRO535 sequence shown in Figure 12 (SEQ ID NO:12) evidences the presence of a variety of important polypeptide domains as shown in Figure 12, wherein the locations given for those important polypeptide domains are approximate as described above. Analysis of the full-length PRO535 sequence evidences the presence of the following features: a signal peptide from about amino acid 1 to about amino acid 25; a transmembrane domain from about amino acid 155 to about amino acid 174; a potential N-glycosylation site from about amino acid 196 to about amino acid 200; an N-myristoylation site from about amino acid 95 to about amino acid 101; an amidation site from about amino acid 119 to about amino acid 123; and FKBP-type peptidyl-prolyl cis-trans isomer signature 1 sequences from about amino acid 62 to about amino acid 78, from about amino acid 87 to about amino acid 124 and from about amino acid 128 to about amino acid 142. Clone DNA49143-1429 has been deposited with ATCC on June 23, 1998 and is assigned ATCC deposit no. 203013.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using the ALIGN-2 sequence alignment

analysis of the full-length sequence shown in Figure 12 (SEQ ID NO:12), evidenced sequence identity between the PRO535 amino acid sequence and the following Dayhoff sequences: S71237, P_R93551, P_R28980, S71238, FKB2_HUMAN, CELC05C8_1, S55383, S72485, CELC50F2_6 and S75144.

EXAMPLE 9

Isolation of cDNA Clones Encoding Human PRO819

Use of the signal sequence algorithm described in Example 2 above allowed identification of an EST cluster sequence from the Incyte database, designated herein as 49605. This EST 49605 cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, Methods in Enzymology, 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases, 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA56015.

In light of an observed sequence homology between the DNA56015 consensus sequence and an EST sequence encompassed within clone no. H65785 from the Merck database, clone H65785 was purchased and the cDNA insert was obtained and sequenced. It was found herein that that insert encoded a full-length protein. The sequence of this cDNA insert is shown in Figure 13 (SEQ ID NO:13) and is herein designated DNA57695-1340.

Clone DNA57695-1340 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 46-48 and ending at the stop codon at nucleotide positions 202-204 (Figure 13). The predicted polypeptide precursor is 52 amino acids long (Figure 14; SEQ ID NO:14). The full-length PRO819 protein shown in Figure 14 has an estimated molecular weight of about 5,216 daltons and a pI of about 4.67.

Analysis of the full-length PRO819 sequence shown in Figure 14 (SEQ ID NO:14) evidences the presence of a variety of important polypeptide domains as shown in Figure 14, wherein the locations given for those important polypeptide domains are approximate as described above. Analysis of the full-length PRO819 sequence evidences the presence of the following features: a signal peptide from about amino acid 1 to about amino acid 24; a potential N-myristoylation site from about amino acid 2 to about amino acid 8; and a region having homology to immunoglobulin light chain from about amino acid 5 to about amino acid 33. Clone DNA57695-1340 has been deposited with ATCC on June 23, 1998 and is assigned ATCC deposit no. 203006.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using the ALIGN-2 sequence alignment analysis of the full-length sequence shown in Figure 14 (SEQ ID NO:14), evidenced sequence identity between the PRO819 amino acid sequence and the following Dayhoff sequences: HSU03899_1, HUMIGLITEB_1, VG28_HSVSA, AF031522_1, PAD1_YEAST and AF045484_1.

EXAMPLE 10

Isolation of cDNA Clones Encoding Human PRO1002

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in

Example 1 above. This consensus sequence is herein designated <Consen0257>.

Incye clone 197165 was identified and the clone was further sequenced based on the results discovered herein. The 197165 clone originated from a breast tumor tissue library, BRSTTUT13.

DNA sequencing of the clone described above gave the full-length DNA sequence for PRO1002 [herein designated as UNQ486 (DNA59208-1373)] (SEQ ID NO:15) and the derived protein sequence for PRO1002.

The entire nucleotide sequence of UNQ486 (DNA59208-1373) is shown in Figure 15 (SEQ ID NO:15). Clone UNQ486 (DNA59208-1373) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 91-93 and ending at the stop codon at nucleotide positions 766-768 (Figure 15). The predicted polypeptide precursor is 225 amino acids long (Figure 16; SEQ ID NO:16). The full-length PRO1002 protein shown in Figure 16 has an estimated molecular weight of about 25,447 daltons and a pI of about 4.79. Clone UNQ486 (DNA59208-1373) has been deposited with the ATCC on May 20, 1998 and is assigned an ATCC deposit no. 209881. Regarding the sequence, it is understood that the deposited clone contains the correct sequence, and the sequences provided herein are based on known sequencing techniques.

Analysis of the amino acid sequence of the full-length PRO1002 polypeptide suggests that portions of it possess significant homology to MBP, thereby indicating that PRO1002 may be a novel cytostimulant and/or toxin.

Still analyzing the amino acid sequence of SEQ ID NO:16, the putative signal peptide is at about amino acids 1-17 of SEQ ID NO:16. N-myristoylation sites are about amino acids 13-19, 103-109, 134-140, 164-170, 180-186, 191-197, 194-200, 196-202, and 198-204. The C-type lectin domain is at about amino acids 200-224 of SEQ ID NO:16. The corresponding nucleotides can be routinely determined given the sequences provided herein.

20

EXAMPLE 11

Isolation of cDNA clones Encoding Human PRO1308

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in Example 1 above. This consensus sequence is herein designated <Consen0616>. In addition, the Consen0616 consensus sequence was extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above. The extended consensus sequence is designated herein as "DNA35726".

Based on the DNA35726 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO1308. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al.*, Current Protocols in Molecular Biology, *supra*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

The following PCR primers (forward and reverse) were synthesized:
forward PCR primers:

5'-TCCTGTGAGCACGTGGTGTG-3' (35726.f1; SEQ ID NO:53)
 5'-GGGTGGGATAGACCTGCG-3' (35726.f2; SEQ ID NO:54)
 5'-AAGGCCAAGAAGGCTGCC-3' (35726.f3; SEQ ID NO:55)
 5'-CCAGGCCTGCAGACCCAG-3' (35726.f4; SEQ ID NO:56)

5 reverse PCR primers:

5'-CTTCCTCAGTCCTTCCAGGATATC-3' (35726.r1; SEQ ID NO:57)
 5'-AAGCTGGATATCCTCCGTGTTGTC-3' (35726.r2; SEQ ID NO:58)
 5'-CCTGAAGAGGCATGACTGCTTTTCTCA-3' (35726.r16.mm; SEQ ID NO:59)
 5'-GGGGATAAACCTATTAATTATTGCTAC-3' (35726.r15.mm; SEQ ID NO:60).

- 10 Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA35726 sequence which had the following nucleotide sequence:

hybridization probe:

5'-AACGTCACCTACATCTCCTCGTGCCACATGCGCCAGGCCACCTG-3' (35726.p1; (SEQ ID NO:61)).

- 15 In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO1308 gene using the probe oligonucleotide and one of the PCR primers.

- 20 RNA for construction of the cDNA libraries was isolated from a human SK-Lu-1 adenocarcinoma cell line. The cDNA libraries used to isolated the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; *see*, Holmes *et al.*, Science, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

- 25 DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO1308 (designated herein as DNA62306-1570 [Figure 17, SEQ ID NO:17]; and the derived protein sequence for PRO1308.

- 30 The entire coding sequence of PRO1308 is shown in Figure 17 (SEQ ID NO:17). Clone DNA62306 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 17-19, and an apparent stop codon at nucleotide positions 806-808. The predicted polypeptide precursor is 263 amino acids long. The full-length PRO1308 protein shown in Figure 18 has an estimated molecular weight of about 27,663 daltons and a pI of about 6.77. Additional features include a signal peptide at about amino acids 1-20, potential N-glycosylation sites at about amino acids 73-77 and 215-219, and regions of homology with osteonectin domains at about amino acids 97-130 and 169-202.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment

analysis of the full-length sequence shown in Figure 18 (SEQ ID NO:18), revealed significant homology between the PRO1308 amino acid sequence and Dayhoff sequence S55369. Homology was also revealed between the PRO1308 amino acid sequence and the following Dayhoff sequences: FSA_HUMAN, P_R20063, CELT13C2_1, AGRI_RAT, p_W09406, G01639, SC1_RAT, S60062, S51362, and IOV7_CHICK.

- 5 Clone DNA62306 (UNQ674), designated as DNA62306-1570 has been deposited with ATCC on September 9, 1998 and is assigned ATCC deposit no. 203254.

EXAMPLE 12

Isolation of cDNA Clones Encoding Human PRO1304

A consensus DNA sequence was assembled relative to other EST sequences using phrap as described in Example 1 above. This consensus sequence is designated herein as DNA35745. Based on the DNA35745 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO1304.

PCR primers (forward and reverse) were synthesized:

forward PCR primer (35745.f1):

- 15 5'-GTGTTCTGCTGGAGCCGATGCC-3' (SEQ ID NO:62)

forward PCR primer (35745.f2):

- 5'-GACATGGACAATGACAGG-3' (SEQ ID NO:63)

forward PCR primer (35745.f3):

- 5'-CCTTTCAGGATGTAGGAG-3' (SEQ ID NO:64)

- 20 forward PCR primer (35745.f4):

5'-GATGTCTGCCACCCCAAG-3' (SEQ ID NO:65)

reverse PCR primer (35745.r1):

5'-GCATCCTGATATGACTTGTCACGTGGC-3' (SEQ ID NO:66)

reverse PCR primer (35745.r2):

- 25 5'-TACAAGAGGGAAGAGGAGTTGCAC-3' (SEQ ID NO:67)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA35745 sequence which had the following nucleotide sequence:

hybridization probe (35745.p1):

5'-GCCCATATGACGGCTACCTGGCTAAAGACGGCTCGAAATTCTACTGCAGCC-3' (SEQ ID NO:68)

- 30 In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO1304 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human ovary tissue.

DNA sequencing of the isolated clones isolated as described above gave the full-length DNA sequence for DNA65406-1567 [Figure 19, SEQ ID NO:19]; and the derived protein sequence for PRO1304.

The entire coding sequence of DNA65406-1567 is included in Figure 19 (SEQ ID NO:19). Clone DNA65406-1567 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 23-25, and an apparent stop codon at nucleotide positions 689-691. The predicted polypeptide precursor is 222 amino acids long, and has an estimated molecular weight of about 25,794 daltons and a pI of about 6.24. Analysis of the full-length PRO1304 sequence shown in Figure 20 (SEQ ID NO:20) evidences the presence of a variety of important polypeptide domains, wherein the locations given for those important polypeptide domains are approximate as described above. Analysis of the full-length PRO1304 polypeptide shown in Figure 20 evidences the presence of the following: an endoplasmic reticulum targeting sequence from about amino acid 219 to about amino acid 224; an N-glycosylation site from about amino acid 45 to about amino acid 49; FKBP-type peptidyl-prolyl cis-trans isomerase homology blocks from about amino acid 87 to about amino acid 124, and from about amino acid 129 to about amino acid 143; an EF-hand calcium-binding domain protein homology block from about amino acid 202 to about amino acid 215 and from about amino acid 195 to about amino acid 215; a tyrosine kinase phosphorylation site from about amino acid 183 to about amino acid 190; and N-myristoylation sites from about amino acid 19 to about amino acid 25 and from about amino acid 95 to about amino acid 101. Clone DNA65406-1567 has been deposited with the ATCC on September 15, 1998 and is assigned ATCC deposit no. 203219.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 20 (SEQ ID NO:20), evidenced significant homology between the PRO1304 amino acid sequence and the following Dayhoff sequences: AF040252_1, P_R28980, S71238, CELC05C8_1, VFU52045_1, S75144, FKB3_BOVIN, CELC50F2_6, CELB0511_12, and P_R41781.

The DNA65406-1567 sequence was also obtained by isolating and sequencing the insert of Incyte EST clone no. 2813577.

EXAMPLE 13

Stimulation of Heart Neonatal Hypertrophy (Assay #1)

This assay is designed to measure the ability of PRO polypeptides to stimulate hypertrophy of neonatal heart. PRO polypeptides testing positive in this assay are expected to be useful for the therapeutic treatment of various cardiac insufficiency disorders.

Cardiac myocytes from 1-day old Harlan Sprague Dawley rats were obtained. Cells ($180 \mu\text{L}$ at $7.5 \times 10^4/\text{ml}$, serum <0.1%, freshly isolated) are added on day 1 to 96-well plates previously coated with DMEM/F12 + 4% FCS. Test samples containing the test PRO polypeptide are added directly to wells on day 2 in $20 \mu\text{L}$ volumes. Cells are stained with crystal violet after an additional two days and scored visually by the next day. Incubator conditions are critical and require 5% CO_2 .

Activity reference: phenylephrine at 1-100 μM , PGF-2 alpha at 0.1-1.0 μM , endothelin-1 at 1-10 nM, CT1 (LIF) at 1-10 nM. No PBS is included, since Ca concentration is critical for assay response. Assay media included: DMEM/F12 (with 2.44 gm bicarbonate), 10 $\mu\text{g}/\text{ml}$ transferrin, 1 $\mu\text{g}/\text{ml}$ insulin, 1 $\mu\text{g}/\text{ml}$ aprotinin, 2 mmol/L

glutamine, 100 U/ml penicillin G, 100 μ g/ml streptomycin. Protein buffer containing mannitol (4%) gave a positive signal (score 3.5) at 1/10 (0.4%) and 1/100 (0.04%), but not at 1/1000 (0.004%). Therefore, the test sample buffer containing mannitol is not run. A secondary assay consists of measuring the ANP levels (ng/ml) by ELISA in conditioned media from the cells. An increase in the ANP message can be measured by PCR from cells after a few hours.

Results are assessed by visually observing cell size: a score = 3.5 or greater is considered positive for conditioned media; a score of 3.0 or greater is considered positive for purified protein.

PRO183 was observed to stimulate neonatal heart hypertrophy and exhibited a positive score in this assay compared with positive controls.

EXAMPLE 14

Stimulation of Endothelial Cell Proliferation (Assay #8)

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to stimulate adrenal cortical capillary endothelial cell (ACE) growth. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of conditions or disorders where angiogenesis would be beneficial including, for example, wound healing, and the like (as would agonists of these PRO polypeptides). Antagonists of the PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of cancerous tumors.

Bovine adrenal cortical capillary endothelial (ACE) cells (from primary culture, maximum of 12-14 passages) were plated in 96-well plates at 500 cells/well per 100 microliter. Assay media included low glucose DMEM, 10% calf serum, 2 mM glutamine, and 1X penicillin/streptomycin/fungizone. Control wells included the following: (1) no ACE cells added; (2) ACE cells alone; (3) ACE cells plus VEGF (5 ng/ml); and (4) ACE cells plus FGF (5ng/ml). The control or test sample, (in 100 microliter volumes), was then added to the wells (at dilutions of 1%, 0.1% and 0.01%, respectively). The cell cultures were incubated for 6-7 days at 37°C/5% CO₂. After the incubation, the media in the wells was aspirated, and the cells were washed 1X with PBS. An acid phosphatase reaction mixture (100 microliter; 0.1M sodium acetate, pH 5.5, 0.1% Triton X-100, 10 mM p-nitrophenyl phosphate) was then added to each well. After a 2 hour incubation at 37°C, the reaction was stopped by addition of 10 microliters 1N NaOH. Optical density (OD) was measured on a microplate reader at 405 nm.

The activity of a PRO polypeptide was calculated as the fold increase in proliferation (as determined by the acid phosphatase activity, OD 405 nm) relative to (1) cell only background, and (2) relative to maximum stimulation by VEGF. VEGF (at 3-10 ng/ml) and FGF (at 1-5 ng/ml) were employed as an activity reference for maximum stimulation. Results of the assay were considered "positive" if the observed stimulation was \geq 50% increase over background. VEGF (5 ng/ml) control at 1% dilution gave 1.24 fold stimulation; FGF (5 ng/ml) control at 1% dilution gave 1.46 fold stimulation.

PRO1710 tested positive in this assay.

EXAMPLE 15

Inhibition of Vascular Endothelial Growth Factor (VEGF) Stimulated Proliferation of Endothelial Cell Growth

(Assay #9)

The ability of various PRO polypeptides to inhibit VEGF stimulated proliferation of endothelial cells was tested. Polypeptides testing positive in this assay are useful for inhibiting endothelial cell growth in mammals where such an effect would be beneficial, e.g., for inhibiting tumor growth.

5 Specifically, bovine adrenal cortical capillary endothelial cells (ACE) (from primary culture, maximum of 12-14 passages) were plated in 96-well plates at 500 cells/well per 100 microliter. Assay media included low glucose DMEM, 10% calf serum, 2 mM glutamine, and 1X penicillin/streptomycin/fungizone. Control wells included the following: (1) no ACE cells added; (2) ACE cells alone; (3) ACE cells plus 5 ng/ml FGF; (4) ACE cells plus 3 ng/ml VEGF; (5) ACE cells plus 3 ng/ml VEGF plus 1 ng/ml TGF-beta; and (6) ACE cells plus 3 ng/ml VEGF plus
10 5 ng/ml LIF. The test samples, poly-his tagged PRO polypeptides (in 100 microliter volumes), were then added to the wells (at dilutions of 1%, 0.1% and 0.01%, respectively). The cell cultures were incubated for 6-7 days at 37°C/5% CO₂. After the incubation, the media in the wells was aspirated, and the cells were washed 1X with PBS. An acid phosphatase reaction mixture (100 microliter; 0.1M sodium acetate, pH 5.5, 0.1% Triton X-100, 10 mM p-nitrophenyl phosphate) was then added to each well. After a 2 hour incubation at 37°C, the reaction was stopped
15 by addition of 10 microliters 1N NaOH. Optical density (OD) was measured on a microplate reader at 405 nm.

The activity of PRO polypeptides was calculated as the percent inhibition of VEGF (3 ng/ml) stimulated proliferation (as determined by measuring acid phosphatase activity at OD 405 nm) relative to the cells without stimulation. TGF-beta was employed as an activity reference at 1 ng/ml, since TGF-beta blocks 70-90% of VEGF-stimulated ACE cell proliferation. The results are indicative of the utility of the PRO polypeptides in cancer therapy
20 and specifically in inhibiting tumor angiogenesis. Numerical values (relative inhibition) are determined by calculating the percent inhibition of VEGF stimulated proliferation by the PRO polypeptides relative to cells without stimulation and then dividing that percentage into the percent inhibition obtained by TGF-β at 1 ng/ml which is known to block 70-90% of VEGF stimulated cell proliferation. The results are considered positive if the PRO polypeptide exhibits 30% or greater inhibition of VEGF stimulation of endothelial cell growth (relative inhibition
25 30% or greater).

PRO183 and PRO202 tested positive in this assay.

EXAMPLE 16Induction of c-fos in Endothelial Cells (Assay #34)

This assay is designed to determine whether PRO polypeptides show the ability to induce c-fos in endothelial
30 cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of conditions or disorders where angiogenesis would be beneficial including, for example, wound healing, and the like (as would agonists of these PRO polypeptides). Antagonists of the PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of cancerous tumors.

Human venous umbilical vein endothelial cells (HUVEC, Cell Systems) in growth media (50% Ham's F12 w/o GHT: low glucose, and 50% DMEM without glycine: with NaHCO₃, 1% glutamine, 10 mM HEPES, 10%
35 FBS, 10 ng/ml bFGF) were plated on 96-well microtiter plates at a cell density of 1x10⁴ cells/well. The day after plating, the cells were starved by removing the growth media and treating the cells with 100 μl/well test samples

and controls (positive control = growth media; negative control = Protein 32 buffer = 10 mM HEPES, 140 mM NaCl, 4% (w/v) mannitol, pH 6.8). The cells were incubated for 30 minutes at 37°C, in 5% CO₂. The samples were removed, and the first part of the bDNA kit protocol (Chiron Diagnostics, cat. #6005-037) was followed, where each capitalized reagent/buffer listed below was available from the kit.

5 Briefly, the amounts of the TM Lysis Buffer and Probes needed for the tests were calculated based on information provided by the manufacturer. The appropriate amounts of thawed Probes were added to the TM Lysis Buffer. The Capture Hybridization Buffer was warmed to room temperature. The bDNA strips were set up in the metal strip holders, and 100 µl of Capture Hybridization Buffer was added to each b-DNA well needed, followed by incubation for at least 30 minutes. The test plates with the cells were removed from the incubator, and the media
10 was gently removed using the vacuum manifold. 100 µl of Lysis Hybridization Buffer with Probes were quickly pipetted into each well of the microtiter plates. The plates were then incubated at 55°C for 15 minutes. Upon removal from the incubator, the plates were placed on the vortex mixer with the microtiter adapter head and vortexed on the #2 setting for one minute. 80 µl of the lysate was removed and added to the bDNA wells containing the Capture Hybridization Buffer, and pipetted up and down to mix. The plates were incubated at 53°C for at least
15 16 hours.

On the next day, the second part of the bDNA kit protocol was followed. Specifically, the plates were removed from the incubator and placed on the bench to cool for 10 minutes. The volumes of additions needed were calculated based upon information provided by the manufacturer. An Amplifier Working Solution was prepared by making a 1:100 dilution of the Amplifier Concentrate (20 fm/µl) in AL Hybridization Buffer. The hybridization
20 mixture was removed from the plates and washed twice with Wash A. 50 µl of Amplifier Working Solution was added to each well and the wells were incubated at 53°C for 30 minutes. The plates were then removed from the incubator and allowed to cool for 10 minutes. The Label Probe Working Solution was prepared by making a 1:100 dilution of Label Concentrate (40 pmoles/µl) in AL Hybridization Buffer. After the 10-minute cool-down period, the amplifier hybridization mixture was removed and the plates were washed twice with Wash A. 50 µl of Label
25 Probe Working Solution was added to each well and the wells were incubated at 53°C for 15 minutes. After cooling for 10 minutes, the Substrate was warmed to room temperature. Upon addition of 3 µl of Substrate Enhancer to each ml of Substrate needed for the assay, the plates were allowed to cool for 10 minutes, the label hybridization mixture was removed, and the plates were washed twice with Wash A and three times with Wash D. 50 µl of the Substrate Solution with Enhancer was added to each well. The plates were incubated for 30 minutes
30 at 37°C and RLU was read in an appropriate luminometer.

The replicates were averaged and the coefficient of variation was determined. The measure of activity of the fold increase over the negative control (Protein 32/HEPES buffer described above) value was indicated by chemiluminescence units (RLU). The results are considered positive if the PRO polypeptide exhibits at least a two-fold value over the negative buffer control. Negative control = 1.00 RLU at 1.00% dilution. Positive control =
35 8.39 RLU at 1.00% dilution.

PRO861 tested positive in this assay.

EXAMPLE 19

Enhancement of Heart Neonatal Hypertrophy Induced by F2a (Assay #37)

This assay is designed to measure the ability of PRO polypeptides to stimulate hypertrophy of neonatal heart. PRO polypeptides testing positive in this assay are expected to be useful for the therapeutic treatment of various cardiac insufficiency disorders.

- 5 Cardiac myocytes from 1-day old Harlan Sprague Dawley rats were obtained. Cells ($180 \mu\text{l}$ at $7.5 \times 10^4/\text{ml}$, serum $<0.1\%$, freshly isolated) are added on day 1 to 96-well plates previously coated with DMEM/F12 + 4% FCS. Test samples containing the test PRO polypeptide ($20 \mu\text{l}/\text{well}$) are added directly to the wells on day 1. PGF ($20 \mu\text{l}/\text{well}$) is then added on day 2 at a final concentration of 10^{-6} M. The cells are then stained on day 4 and visually scored on day 5. Visual scores are based on cell size, wherein cells showing no increase in size as compared to negative controls are scored 0.0, cells showing a small to moderate increase in size as compared to negative controls are scored 1.0 and cells showing a large increase in size as compared to negative controls are scored 2.0. A score of 1.0 or greater is considered positive.

- 15 No PBS is included, since calcium concentration is critical for assay response. Plates are coated with DMEM/F12 plus 4% FCS ($200 \mu\text{l}/\text{well}$). Assay media included: DMEM/F12 (with 2.44 gm bicarbonate), $10 \mu\text{g}/\text{ml}$ transferrin, $1 \mu\text{g}/\text{ml}$ insulin, $1 \mu\text{g}/\text{ml}$ aprotinin, 2 mmol/L glutamine, 100 U/ml penicillin G, $100 \mu\text{g}/\text{ml}$ streptomycin. Protein buffer containing mannitol (4%) gave a positive signal (score 3.5) at 1/10 (0.4%) and 1/100 (0.04%), but not at 1/1000 (0.004%). Therefore the test sample buffer containing mannitol is not run.

PRO183 and PRO202 tested positive in this assay.

EXAMPLE 18

- 20 Induction of Endothelial Cell Apoptosis (Assay #73)

The ability of PRO polypeptides to induce apoptosis in endothelial cells was tested in human venous umbilical vein endothelial cells (HUVEC, Cell Systems). A positive test in the assay is indicative of the usefulness of the polypeptide in therapeutically treating tumors as well as vascular disorders where inducing apoptosis of endothelial cells would be beneficial.

- 25 The cells were plated on 96-well microtiter plates (Amersham Life Science, cytostar-T scintillating microplate, RPNQ160, sterile, tissue-culture treated, individually wrapped), in 10% serum (CSG-medium, Cell Systems), at a density of 2×10^4 cells per well in a total volume of $100 \mu\text{l}$. On day 2, test samples containing the PRO polypeptide were added in triplicate at dilutions of 1%, 0.33% and 0.11%. Wells without cells were used as a blank and wells with cells only were used as a negative control. As a positive control 1:3 serial dilutions of $50 \mu\text{l}$ of a 30 3x stock of staurosporine were used. The ability of the PRO polypeptide to induce apoptosis was determined by processing of the 96 well plates for detection of Annexin V, a member of the calcium and phospholipid binding proteins, to detect apoptosis.

- 35 0.2 ml Annexin V - Biotin stock solution ($100 \mu\text{g}/\text{ml}$) was diluted in 4.6 ml $2 \times \text{Ca}^{2+}$ binding buffer and 2.5% BSA (1:25 dilution). $50 \mu\text{l}$ of the diluted Annexin V - Biotin solution was added to each well (except controls) to a final concentration of $1.0 \mu\text{g}/\text{ml}$. The samples were incubated for 10-15 minutes with Annexin-Biotin prior to direct addition of ^{35}S -Streptavidin. ^{35}S -Streptavidin was diluted in $2 \times \text{Ca}^{2+}$ Binding buffer, 2.5% BSA and was added to all wells at a final concentration of 3×10^4 cpm/well. The plates were then sealed, centrifuged at 1000

rpm for 15 minutes and placed on orbital shaker for 2 hours. The analysis was performed on a 1450 Microbeta Trilux (Wallac). Percent above background represents the percentage amount of counts per minute above the negative controls. Percents greater than or equal to 30% above background are considered positive. PRO198 gave positive results (induced endothelial cell apoptosis) in the above described assay.

5

EXAMPLE 19

Enhancement of Heart Neonatal Hypertrophy Induced by LIF + ET-1 (Assay #75)

This assay is designed to measure the ability of PRO polypeptides to stimulate hypertrophy of neonatal heart. PRO polypeptides testing positive in this assay are expected to be useful for the therapeutic treatment of various cardiac insufficiency disorders.

10 Cardiac myocytes from 1-day old Harlan Sprague Dawley rats were obtained. Cells ($180 \mu\text{l}$ at $7.5 \times 10^4/\text{ml}$, serum $<0.1\%$, freshly isolated) are added on day 1 to 96-well plates previously coated with DMEM/F12 + 4% FCS. Test samples containing the test PRO polypeptide are added directly to wells on day 2 in $20 \mu\text{L}$ volumes. LIF and ET are added on Day 3. Cells are stained with crystal violet after an additional two days and scored visually by the next day. Incubator conditions are critical and require $5\% \text{CO}_2$.

15 Activity reference: CT1 (LIF) at 0.1 nM , 0.01 nM ; endothelin-1 at 1.0 nM . No PBS is included, since Ca concentration is critical for assay response. Assay media included: DMEM/F12 (with 2.44 gm bicarbonate), $10 \mu\text{g}/\text{ml}$ transferrin, $1 \mu\text{g}/\text{ml}$ insulin, $1 \mu\text{g}/\text{ml}$ aprotinin, 2 mmol/L glutamine, $100 \text{ U}/\text{ml}$ penicillin G, $100 \mu\text{g}/\text{ml}$ streptomycin. Protein buffer containing mannitol (4%) gave a positive signal (score 3.5) at $1/10$ (0.4%) and $1/100$ (0.04%), but not at $1/1000$ (0.004%). Therefore, the test sample buffer containing mannitol is not run. A secondary
20 assay consists of measuring the ANP levels (ng/ml) by ELISA in conditioned media from the cells. An increase in the ANP message can be measured by PCR from cells after a few hours.

Results are assessed by visually observing cell size: a score = 3.5 or greater is considered positive for conditioned media; a score of 3.0 or greater is considered positive for purified protein.

PRO200 and PRO882 purified polypeptides were observed to stimulate neonatal heart hypertrophy and
25 exhibited positive scores in this assay compared with positive controls.

EXAMPLE 20

Detection of Endothelial Cell Apoptosis (FACS) (Assay #96)

The ability of PRO polypeptides of the present invention to induce apoptosis in endothelial cells was tested in human venous umbilical vein endothelial cells (HUVEC, Cell Systems) in gelatinized T175 flasks using
30 HUVEC cells below passage 10. PRO polypeptides testing positive in this assay are expected to be useful for therapeutically treating conditions where apoptosis of endothelial cells would be beneficial including, for example, the therapeutic treatment of tumors.

On day one, the cells were split [$420,000$ cells per gelatinized 6 cm dishes - (11×10^3 cells/ cm^2 Falcon, Primaria)] and grown in media containing serum (CS-C, Cell System) overnight or for 16 hours to 24 hours.

35 On day 2, the cells were washed 1x with 5 ml PBS ; 3 ml of 0% serum medium was added with VEGF ($100 \text{ ng}/\text{ml}$); and $30 \mu\text{l}$ of the PRO test compound (final dilution 1%) or 0% serum medium (negative control)

was added. The mixtures were incubated for 48 hours before harvesting.

The cells were then harvested for FACS analysis. The medium was aspirated and the cells washed once with PBS. 5 ml of 1 x trypsin was added to the cells in a T-175 flask, and the cells were allowed to stand until they were released from the plate (about 5-10 minutes). Trypsinization was stopped by adding 5 ml of growth media. The cells were spun at 1000 rpm for 5 minutes at 4°C. The media was aspirated and the cells were resuspended in 10 ml of 10% serum complemented medium (Cell Systems), 5 µl of Annexin-FITC (BioVison) added and chilled tubes were submitted for FACS. A positive result was determined to be enhanced apoptosis in the PRO polypeptide treated samples as compared to the negative control.

PRO1002 and PRO1304 tested positive in this assay.

EXAMPLE 21

Fibroblast (BHK-21) Proliferation (Assay #98)

This assay shows that certain PRO polypeptides of the invention act to induce proliferation of mammalian fibroblast cells in culture and, therefore, function as useful growth factors in mammalian systems. The assay is performed as follows. BHK-21 fibroblast cells plated in standard growth medium at 2500 cells/well in a total volume of 100 µl. The PRO polypeptide, β-FGF (positive control) or nothing (negative control) are then added to the wells in the presence of 1 µg/ml of heparin for a total final volume of 200 µl. The cells are then incubated at 37°C for 6 to 7 days. After incubation, the media is removed, the cells are washed with PBS and then an acid phosphatase substrate reaction mixture (100 µl/well) is added. The cells are then incubated at 37°C for 2 hours. 10 µl per well of 1N NaOH is then added to stop the acid phosphatase reaction. The plates are then read at OD 405nm. A positive in the assay is acid phosphatase activity which is at least 50% above the negative control.

PRO356 and PRO1308 tested positive in this assay.

EXAMPLE 22

Inhibition of bFGF Stimulated Proliferation of Endothelial Cell Growth (HUVEC) (Assay #104)

The ability of various PRO polypeptides to inhibit bFGF stimulated proliferation of endothelial cells was tested. Polypeptides testing positive in this assay are useful for inhibiting endothelial cell growth in mammals where such an effect would be beneficial, e.g., for inhibiting tumor growth.

Specifically, HUVEC cells in Media 199 (Sigma) w/2% FBS (Hyclone) were plated in 96-well plates at 500 cells/well per 100 microliter and left overnight. Growth Media included: EGM plus 1x Pen Strep plus 1x L-Glutamine plus 10% FBS Assay Media: Media 199 plus 2% FBS plus 50 ng/ml bFGF. On day 2, proteins were diluted at 3 concentrations (1%, 0.1%, 0.01%) in the presence of bFGF (50 ng/ml) and added to the cells. The following steps were done as described in the instruction booklet for the Boehringer Mannheim BrdU Cell Proliferation ELISA (Cat No. 1 637 229). On day 3, the cells were labeled with BrdU (final concentration 10 µM). On day 4, the cells were fixed and the DNA was denatured by adding FixDenat for 30 minutes. The FixDenat was removed and the anti-BrdU-POD was added after 2 hours. The plate was then washed and the substrate was added. After allowing the color to develop for 5 minutes, the reaction was quenched with 1 M H₂SO₄. The absorbance (OD) was measured on a microplate reader at 405 nm.

The activity of PRO polypeptides to inhibit bFGF stimulation of HUVEC proliferation was measured against the control cells (bFGF alone). Any test sample that gave an absorbance reading significantly below that of bFGF was considered as a positive result in this assay. The results are indicative of the utility of the PRO polypeptides in cancer therapy and specifically in inhibiting tumor angiogenesis.

5 PRO175, PRO364, PRO535, PRO819 and PRO879 polypeptides tested positive in this assay:

EXAMPLE 23

Induction of Endothelial Cell Apoptosis (ELISA) (Assay #109)

The ability of PRO polypeptides to induce apoptosis in endothelial cells was tested in human venous umbilical vein endothelial cells (HUVEC, Cell Systems) using a 96-well format, in 0% serum media supplemented with 100
10 ng/ml VEGF, 0.1% BSA, 1X penn/strep. The 96-well plates used were manufactured by Falcon (No. 3072). Coating of 96 well plates were prepared by allowing gelatinization to occur for >30 minutes with 100 μ l of 0.2% gelatin in PBS solution. The gelatin mix was aspirated thoroughly before plating HUVE cells at a final concentration of 2×10^4 cells/ml in 10% serum containing medium - 100 μ l volume per well. The cells were grown for 24 hours before adding test samples containing the PRO polypeptide of interest.

15 To all wells, 100 μ l of 0% serum media (Cell Systems) complemented with 100 ng/ml VEGF, 0.1% BSA, 1X penn/strep. was added. Test samples containing PRO polypeptides were added in triplicate at dilutions of 1%, 0.33% and 0.11%. Wells without cells were used as a blank and wells with cells only were used as a negative control. As a positive control 1:3 serial dilutions of 50 μ l of a 3x stock of staurosporine were used. The cells were incubated for 24 to 35 hours prior to ELISA.

20 ELISA was used to determine levels of apoptosis preparing solutions according to the Boehringer Manual [Boehringer, Cell Death Detection ELISA plus, Cat No. 1 920 685]. Sample preparations: 96 well plates were spun down at 1 krpm for 10 minutes (200g); the supernatant was removed by fast inversion, placing the plate upside down on a paper towel to remove residual liquid. To each well, 200 μ l of 1X Lysis buffer was added and incubation allowed at room temperature for 30 minutes without shaking. The plates were spun down for 10 minutes
25 at 1 krpm, and 20 μ l of the lysate (cytoplasmic fraction) was transferred into streptavidin coated MTP. 80 μ l of immunoreagent mix was added to the 20 μ l lysate in each well. The MTP was covered with adhesive foil and incubated at room temperature for 2 hours by placing it on an orbital shaker (200 rpm). After two hours, the supernatant was removed by suction and the wells rinsed three times with 250 μ l of 1X incubation buffer per well (removed by suction). Substrate solution was added (100 μ l) into each well and incubated on an orbital shaker at
30 room temperature at 250 rpm until color development was sufficient for a photometric analysis (approx. after 10-20 minutes). A 96 well reader was used to read the plates at 405 nm, reference wavelength, 492 nm. The levels obtained for PIN 32 (control buffer) was set to 100%. Samples with levels >130% were considered positive for induction of apoptosis. PRO877 gave positive results as measured by the ELISA assay described above.

EXAMPLE 24

In situ Hybridization

35 *In situ* hybridization is a powerful and versatile technique for the detection and localization of nucleic acid

sequences within cell or tissue preparations. It may be useful, for example, to identify sites of gene expression, analyze the tissue distribution of transcription, identify and localize viral infection, follow changes in specific mRNA synthesis, and aid in chromosome mapping.

In situ hybridization was performed following an optimized version of the protocol by Lu and Gillett, Cell Vision, 1: 169-176 (1994), using PCR-generated ^{33}P -labeled riboprobes. Briefly, formalin-fixed, paraffin-embedded human tissues were sectioned, deparaffinized, deproteinized in proteinase K (20 g/ml) for 15 minutes at 37°C, and further processed for *in situ* hybridization as described by Lu and Gillett, *supra*. A (^{33}P -UTP)-labeled antisense riboprobe was generated from a PCR product and hybridized at 55°C overnight. The slides were dipped in Kodak NTB2™ nuclear track emulsion and exposed for 4 weeks.

^{33}P -Riboprobe synthesis

6.0 μl (125 mCi) of ^{33}P -UTP (Amersham BF 1002, SA<2000 Ci/mmol) were speed-vacuum dried. To each tube containing dried ^{33}P -UTP, the following ingredients were added:

2.0 μl 5x transcription buffer

1.0 μl DTT (100 mM)

2.0 μl NTP mix (2.5 mM: 10 μl each of 10 mM GTP, CTP & ATP + 10 μl H₂O)

1.0 μl UTP (50 μM)

1.0 μl RNasin

1.0 μl DNA template (1 μg)

1.0 μl H₂O

1.0 μl RNA polymerase (for PCR products T3 = AS, T7 = S, usually)

The tubes were incubated at 37°C for one hour. A total of 1.0 μl RQ1 DNase was added, followed by incubation at 37°C for 15 minutes. A total of 90 μl TE (10 mM Tris pH 7.6/1 mM EDTA pH 8.0) was added, and the mixture was pipetted onto DE81 paper. The remaining solution was loaded in a MICROCON-50™ ultrafiltration unit, and spun using program 10 (6 minutes). The filtration unit was inverted over a second tube and spun using program 2 (3 minutes). After the final recovery spin, a total of 100 μl TE was added, then 1 μl of the final product was pipetted on DE81 paper and counted in 6 ml of BIOFLUOR II™.

The probe was run on a TBE/urea gel. A total of 1-3 μl of the probe or 5 μl of RNA Mrk III was added to 3 μl of loading buffer. After heating on a 95°C heat block for three minutes, the gel was immediately placed on ice. The wells of gel were flushed, and the sample was loaded and run at 180-250 volts for 45 minutes. The gel was wrapped in plastic wrap (SARAN™ brand) and exposed to XAR film with an intensifying screen in a -70°C freezer one hour to overnight.

^{33}P -Hybridization

A. *Pretreatment of frozen sections*

The slides were removed from the freezer, placed on aluminum trays, and thawed at room temperature for 5 minutes. The trays were placed in a 55°C incubator for five minutes to reduce condensation. The slides were fixed for 10 minutes in 4% paraformaldehyde on ice in the fume hood, and washed in 0.5 x SSC for 5 minutes, at room temperature (25 ml 20 x SSC + 975 ml SQ H₂O). After deproteinization in 0.5 $\mu\text{g}/\text{ml}$ proteinase K for 10 minutes at 37°C (12.5 μl of 10 mg/ml stock in 250 ml prewarmed RNase-free RNase buffer), the sections were washed

in 0.5 x SSC for 10 minutes at room temperature. The sections were dehydrated in 70%, 95%, and 100% ethanol, 2 minutes each.

B. *Pretreatment of paraffin-embedded sections*

The slides were deparaffinized, placed in SQ H₂O, and rinsed twice in 2 x SSC at room temperature, for 5 minutes each time. The sections were deproteinized in 20 µg/ml proteinase K (500 µl of 10 mg/ml in 250 ml RNase-free RNase buffer; 37°C, 15 minutes) for human embryo tissue, or 8 x proteinase K (100 µl in 250 ml RNase buffer, 37°C, 30 minutes) for formalin tissues. Subsequent rinsing in 0.5 x SSC and dehydration were performed as described above.

C. *Prehybridization*

The slides were laid out in a plastic box lined with Box buffer (4 x SSC, 50% formamide) - saturated filter paper. The tissue was covered with 50 µl of hybridization buffer (3.75 g dextran sulfate + 6 ml SQ H₂O), vortexed, and heated in the microwave for 2 minutes with the cap loosened. After cooling on ice, 18.75 ml formamide, 3.75 ml 20 x SSC, and 9 ml SQ H₂O were added, and the tissue was vortexed well and incubated at 42°C for 1-4 hours.

D. *Hybridization*

1.0 x 10⁶ cpm probe and 1.0 µl tRNA (50 mg/ml stock) per slide were heated at 95°C for 3 minutes. The slides were cooled on ice, and 48 µl hybridization buffer was added per slide. After vortexing, 50 µl ³²P mix was added to 50 µl prehybridization on the slide. The slides were incubated overnight at 55°C.

E. *Washes*

Washing was done for 2x10 minutes with 2xSSC, EDTA at room temperature (400 ml 20 x SSC + 16 ml 0.25 M EDTA, V=4L), followed by RNaseA treatment at 37°C for 30 minutes (500 µl of 10 mg/ml in 250 ml RNase buffer = 20 µg/ml). The slides were washed 2 x10 minutes with 2 x SSC, EDTA at room temperature. The stringency wash conditions were as follows: 2 hours at 55°C, 0.1 x SSC, EDTA (20 ml 20 x SSC + 16 ml EDTA, V=4L).

F. *Oligonucleotides*

In situ analysis was performed on four of the DNA sequences disclosed herein. The oligonucleotides employed for these analyses are as follows:

(1) DNA19355-1150 (PRO175) (TNF motif homolog)

19355 p1:

5'-TCTAATACGACTCACTATAGCTCAGGGGAAGAGCCAAAGA-3' (SEQ ID NO:69)

19355 p2:

5'-TGAATTAACCCTCACTAAAGCAGTGCAATGCAGGGGACTA-3' (SEQ ID NO:70)

(2) DNA29101-1122 (PRO200)

p1:

5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC GGC GGA ATC CAA CCT GAG TAG-3' (SEQ ID NO:71)

p2:

5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA GCG GCT ATC CTC CTG TGC TC-3' (SEQ ID NO:72)

(3) DNA47365-1206 (PRO364) (TNF receptor homolog)

p1:

5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC AAC CCG AGC ATG GCA CAG CAC-3' (SEQ ID NO:73)

p2:

5 5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA TCT CCC AGC CGC CCC TTC TC-3' (SEQ ID NO:74)

(4) DNA28498 (PRO183) (FHF-2)

p1:

5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC CAG CAA AAG AAG CGG TGG TG-3' (SEQ ID NO:75)

p2:

10 5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA TTC AGC ACG CCA GAG ACA CTT-3' (SEQ ID NO:76)

p3:

5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC ATT CCG CTT GCA CAG TGT CCG-3' (SEQ ID NO:77)

p4:

5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA CCA CCA CTC GCA GAC CCA CAG-3' (SEQ ID NO:78)

15 G. *Results*

In situ analysis was performed on the above four DNA sequences disclosed herein. The results from these analyses are as follows:

(1) DNA19355-1150-1 (PRO175) (TNF motif)

A specific positive signal was observed over the population of endothelial cells in the fetal adrenals.

20 Endothelial cells elsewhere were negative.

(2) DNA29101-1122 (PRO200) (VEGF homolog)*Expression in Human Tissues*

In fetal tissues, expression was observed in developing lower limb bones at the edge of the cartilaginous anlage (*i.e.*, around the outside edge). Also, expression was seen in developing tendons, in vascular smooth muscle and in cells embracing developing skeletal muscle myocytes and myotubes. Expression was also observed at the epiphyseal growth plate. Expression was also seen in the marginal sinus of developing lymph nodes. In addition, expression in the thymus was seen in the subcapsular region of the thymic cortex, possibly representing either the subcapsular epithelial cells or the proliferating thymocytes that are found in this region. Expression in the smooth muscle of the trachea was also reported. In the brain cerebral cortex, focal expression occurred in cortical neurons.

30 Expression was also observed in the following tissues: smooth muscle of the small intestine, generalized expression over the thyroid epithelium, liver expression in ductal plate cells, stomach expression in mural smooth muscle, fetal skin expression in the basal layer of squamous epithelium, placenta expression in interstitial cells (in trophoblastic villi) and cord expression in the wall of arteries and vein. The spleen, spinal cord, and the adrenals showed negative results.

Expression patterns suggest that this VEGF homolog may be involved in cell differentiation and/or proliferation. The expression pattern in developing skeletal muscle suggests a possible role in myoblast differentiation and/or proliferation.

Expression in Tumors and Fetal Tissues

5 High expression was observed at the following sites:

Chimp ovary - granulosa cells of maturing follicles, lower intensity signal observed over thecal cells

Chimp parathyroid - high expression over chief cells

Human fetal testis - moderate expression over stromal cells surrounding developing tubules

10 Human fetal lung - high expression over chondrocytes in developing bronchial tree, and low level expression over branching bronchial epithelium

Specific expression was not observed over the renal cell, gastric and colonic carcinomas.

Fetal tissues examined (E12-E16 weeks) included: placenta, umbilical cord, liver, kidney, adrenals, thyroid, lungs, heart, great vessels, esophagus, stomach, small intestine, spleen, thymus, pancreas, brain, eye, spinal cord, 15 body wall, pelvis and lower limb.

Adult tissues examined included: liver, kidney, adrenals, myocardium, aorta, spleen, lymph node, pancreas, lung, skin, cerebral cortex (rm), hippocampus (rm), cerebellum (rm), penis, eye, bladder, gastric carcinoma, colon, colonic carcinoma, chondrosarcoma, acetaminophen induced liver injury and hepatic cirrhosis.

(3) DNA47365-1206 (PRO364) (TNF receptor homolog)

20 In the fetus, there was expression in the fascia lining the anterior surface of the vertebral body. There was also expression over the fetal retina. However, there was low level expression over the fetal neurones. All other tissues were negative.

(4) DNA28498 (PRO183) (FHF-2)

25 Expression was seen over the inner aspect of the fetal retina. Strong expression was seen over the spinal ganglia and over neurones in the anterior horns of the spinal cord of the human fetus. While significant expression was not observed in the human fetal brain, high expression was observed over neurones in rhesus monkey brain, including hippocampal neurones.

EXAMPLE 25

30 Use of PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 as a Hybridization Probe

The following method describes use of a nucleotide sequence encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 as a hybridization probe.

35 DNA comprising the coding sequence of full-length or mature PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879,

PRO882 or PRO1710 (as shown in Figures 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31 and 33, respectively) or a fragment thereof is employed as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants of PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710) in human tissue cDNA libraries or human tissue genomic libraries.

Hybridization and washing of filters containing either library DNAs is performed under the following high-stringency conditions. Hybridization of radiolabeled probe derived from the gene encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding full-length native sequence can then be identified using standard techniques known in the art.

EXAMPLE 26

Expression of Nucleic Acid Encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 in

E. coli

This Example illustrates preparation of an unglycosylated form of PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 by recombinant expression in *E. coli*.

The DNA sequence encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 (SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31 or 33, respectively) is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites that correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar *et al.*, Gene, 2: 95 (1977)), which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR-amplified sequences are then ligated into the vector. The vector will preferably include sequences that encode an antibiotic-resistance gene, a trp promoter, a poly-His leader (including the first six STII codons, poly-His sequence, and enterokinase cleavage site), the region encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710, lambda transcriptional terminator, and an argU gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook *et al.*, *supra*. Transformants are identified by their ability to grow on LB plates and antibiotic-resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger-scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide can then be purified using a metal-chelating column under conditions that allow tight binding of the polypeptide.

PRO183, PRO200, PRO202, PRO364 and PRO1308 were successfully expressed in *E. coli* in a poly-His tagged form by the above procedure.

EXAMPLE 27

Expression of Nucleic Acid Encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 in Mammalian Cells

This Example illustrates preparation of a potentially glycosylated form of PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 by recombinant expression in mammalian cells.

The vector, pRK5 (*see*, EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the DNA encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 using ligation methods such as described in Sambrook *et al.*, *supra*. The resulting vector is called pRK5-(DNA encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710).

In one embodiment, the selected host cells are 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 μ g DNA of pRK5-(DNA encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710) is mixed with about 1 μ g DNA encoding the VA RNA gene (Thimmappaya *et al.*, *Cell*, 31: 543 (1982)) and dissolved in 500 μ l of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M CaCl_2 . To this mixture is added, dropwise, 500 μ l of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM NaPO_4 , and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum-free medium, fresh medium is added, and the cells are incubated for about 5 days.

Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture

medium (alone) or culture medium containing 200 $\mu\text{Ci/ml}$ ^{35}S -cysteine and 200 $\mu\text{Ci/ml}$ ^{35}S -methionine. After a 12-hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of the PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide. The cultures containing transfected cells may undergo further incubation (in serum-free medium) and the medium is tested in selected bioassays.

In an alternative technique, the gene encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 may be introduced into 293 cells transiently using the dextran sulfate method described by Sompayrac *et al.*, Proc. Natl. Acad. Sci., 12: 7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 μg pRK5- (DNA encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710) is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 $\mu\text{g/ml}$ bovine insulin, and 0.1 $\mu\text{g/ml}$ bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing the expressed gene encoding the PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, the gene encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 can be expressed in CHO cells. The pRK5- (DNA encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710) nucleic acid can be transfected into CHO cells using known reagents such as CaPO_4 or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as ^{35}S -methionine. After determining the presence of the PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide, the culture medium may be replaced with serum-free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 can then be concentrated and purified by any selected method.

Epitope-tagged gene encoding the PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide may also be expressed in host CHO cells. The gene encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR

amplification to fuse in frame with a selected epitope tag such as a poly-His tag into a baculovirus expression vector. The gene insert encoding the poly-His-tagged-[PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710] can then be subcloned into a SV40- driven vector containing a selection marker such as DHFR for selection of

5 stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40-driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed gene encoding the poly-His-tagged-[PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710] can then be concentrated and purified by any selected method, such as by Ni^{2+} -chelate affinity chromatography.

- 10 PRO175, PRO198, PRO200, PRO202, PRO356, PRO364, PRO535, PRO861, PRO1304 and PRO1308 were stably expressed in CHO cells by the above described method. In addition, PRO356, PRO364, PRO535 and PRO861 were expressed in CHO cells by a transient procedure.

EXAMPLE 28

- 15 Expression of Nucleic Acid Encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 in

Yeast

- The following method describes recombinant expression of the gene encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877,
- 20 PRO879, PRO882 or PRO1710 in yeast.

- First, yeast expression vectors are constructed for intracellular production or secretion of PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 from the ADH2/GAPDH promoter. DNA encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of the gene encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710. For secretion, DNA encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710
- 30 can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, a native PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 signal peptide or other mammalian signal peptide, or, for example, a yeast alpha-factor or invertase secretory signal/leader sequence, and linker sequences (if needed) for expression of the gene encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710.
- 35 PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue

stain.

Recombinant PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 may further be purified using selected column-chromatography resins.

EXAMPLE 29

Expression of Nucleic Acid Encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 in

Baculovirus-Infected Insect Cells

The following method describes recombinant expression in Baculovirus-infected insect cells.

The sequence coding for PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-His tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the sequence encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 or the desired portion of the coding sequence of PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 [such as the sequence encoding the extracellular domain of a transmembrane protein or the sequence encoding the mature protein if the protein is extracellular] is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfecting the above plasmid and BaculoGold™ virus DNA (PharMingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley *et al.*, Baculovirus Expression Vectors: A Laboratory Manual (Oxford: Oxford University Press (1994)).

Expressed poly-His tagged-[PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710] can then be purified, for example, by Ni²⁺-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert *et al.*, Nature, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 ml Hepes, pH 7.9; 12.5 mM MgCl₂; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.8) and

filtered through a 0.45 μ m filter. A Ni²⁺-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 ml, washed with 25 ml of water and equilibrated with 25 ml of loading buffer. The filtered cell extract is loaded onto the column at 0.5 ml per minute. The column is washed to baseline A₂₈₀ with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% glycerol, pH 6.0), which elutes non-specifically-bound protein. After reaching A₂₈₀ baseline again, the column is developed with a 0 to 500 mM imidazole gradient in the secondary wash buffer. One ml fractions are collected and analyzed by SDS-PAGE and silver staining or Western blot with Ni²⁺-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His₁₀-tagged-[PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710], respectively, are pooled and dialyzed against loading buffer.

Alternatively, purification of the IgG-tagged (or Fc tagged)-[PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710] can be performed using known chromatography techniques, including for instance, Protein A or protein G column chromatography.

While expression was actually performed in a 0.5-2 L scale, it can be readily scaled up for larger (e.g., 8 L) preparations. The proteins are expressed as an IgG construct (immunoadhesin), in which the protein extracellular region is fused to an IgG1 constant region sequence containing the hinge, CH2 and CH3 domains and/or in poly-His tagged forms.

Following PCR amplification, the respective coding sequences are subcloned into a baculovirus expression vector (pb.PH.IgG for IgG fusions and pb.PH.His.c for poly-His tagged proteins), and the vector and Baculogold® baculovirus DNA (Pharmingen) are co-transfected into 105 *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711), using Lipofectin (Gibco BRL). pb.PH.IgG and pb.PH.His are modifications of the commercially available baculovirus expression vector pVL1393 (Pharmingen), with modified polylinker regions to include the His or Fc tag sequences. The cells are grown in Hink's TNM-FH medium supplemented with 10% FBS (Hyclone). Cells are incubated for 5 days at 28°C. The supernatant is harvested and subsequently used for the first viral amplification by infecting Sf9 cells in Hink's TNM-FH medium supplemented with 10% FBS at an approximate multiplicity of infection (MOI) of 10. Cells are incubated for 3 days at 28°C. The supernatant is harvested and the expression of the constructs in the baculovirus expression vector is determined by batch binding of 1 ml of supernatant to 25 ml of Ni²⁺-NTA beads (QIAGEN) for histidine tagged proteins or Protein-A Sepharose CL-4B beads (Pharmacia) for IgG tagged proteins followed by SDS-PAGE analysis comparing to a known concentration of protein standard by Coomassie blue staining.

The first viral amplification supernatant is used to infect a spinner culture (500 ml) of Sf9 cells grown in ESF-921 medium (Expression Systems LLC) at an approximate MOI of 0.1. Cells are incubated for 3 days at 28°C. The supernatant is harvested and filtered. Batch binding and SDS-PAGE analysis are repeated, as necessary, until expression of the spinner culture is confirmed.

The conditioned medium from the transfected cells (0.5 to 3 L) is harvested by centrifugation to remove the cells and filtered through 0.22 micron filters. For the poly-His tagged constructs, the protein construct is purified using a Ni²⁺-NTA column (Qiagen). Before purification, imidazole is added to the conditioned media to a

concentration of 5 mM. The conditioned media is pumped onto a 6 ml Ni²⁺-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column is washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein is subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc containing) constructs of proteins are purified from the conditioned media as follows. The conditioned media is pumped onto a 5 ml Protein A column (Pharmacia) which has been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column is washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein is immediately neutralized by collecting 1 ml fractions into tubes containing 275 ml of 1 M Tris buffer, pH 9. The highly purified protein is subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity of the proteins is verified by SDS polyacrylamide gel (PAGE) electrophoresis and N-terminal amino acid sequencing by Edman degradation.

PRO175, PRO183 and PRO819 were successfully expressed in Baculovirus- infected insect Sf9 cells by the above procedure.

Alternatively, a modified baculovirus procedure may be used incorporating high-5 cells. In this procedure, the DNA encoding the desired sequence is amplified with suitable systems, such as Pfu (Stratagene), or fused upstream (5'-of) of an epitope tag contained with a baculovirus expression vector. Such epitope tags include poly-His tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pIE1-1 (Novagen). The pIE1-1 and pIE1-2 vectors are designed for constitutive expression of recombinant proteins from the baculovirus ie1 promoter in stably-transformed insect cells. The plasmids differ only in the orientation of the multiple cloning sites and contain all promoter sequences known to be important for ie1-mediated gene expression in uninfected insect cells as well as the hr5 enhancer element. pIE1-1 and pIE1-2 include the translation initiation site and can be used to produce fusion proteins. Briefly, the desired sequence or the desired portion of the sequence (such as the sequence encoding the extracellular domain of a transmembrane protein) is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector. For example, derivatives of pIE1-1 can include the Fc region of human IgG (pb.PH.IgG) or an 8 histidine (pb.PH.His) tag downstream (3'-of) the desired sequence. Preferably, the vector construct is sequenced for confirmation.

High-5 cells are grown to a confluency of 50% under the conditions of, 27°C, no CO₂, NO pen/strep. For each 150 mm plate, 30 µg of pIE based vector containing the sequence is mixed with 1 ml Ex-Cell medium (Media: Ex-Cell 401 + 1/100 L-Glu JRH Biosciences #14401-78P (note: this media is light sensitive)), and in a separate tube, 100 µl of CellFectin (CellFECTIN (GibcoBRL #10362-010) (vortexed to mix)) is mixed with 1 ml of Ex-Cell medium. The two solutions are combined and allowed to incubate at room temperature for 15 minutes. 8 ml of Ex-Cell media is added to the 2 ml of DNA/CellFECTIN mix and this is layered on high-5 cells that have been washed once with Ex-Cell media. The plate is then incubated in darkness for 1 hour at room temperature. The DNA/CellFECTIN mix is then aspirated, and the cells are washed once with Ex-Cell to remove excess

CellFECTIN, 30 ml of fresh Ex-Cell media is added and the cells are incubated for 3 days at 28°C. The supernatant is harvested and the expression of the sequence in the baculovirus expression vector is determined by batch binding of 1 ml of supernatant to 25 ml of Ni²⁺-NTA beads (QIAGEN) for histidine tagged proteins or Protein-A Sepharose CL-4B beads (Pharmacia) for IgG tagged proteins followed by SDS-PAGE analysis comparing to a known concentration of protein standard by Coomassie blue staining.

The conditioned media from the transfected cells (0.5 to 3 L) is harvested by centrifugation to remove the cells and filtered through 0.22 micron filters. For the poly-His tagged constructs, the protein comprising the sequence is purified using a Ni²⁺-NTA column (Qiagen). Before purification, imidazole is added to the conditioned media to a concentration of 5 mM. The conditioned media is pumped onto a 6 ml Ni²⁺-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 48°C. After loading, the column is washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein is then subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc containing) constructs of proteins are purified from the conditioned media as follows. The conditioned media is pumped onto a 5 ml Protein A column (Pharmacia) which has been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column is washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein is immediately neutralized by collecting 1 ml fractions into tubes containing 275 ml of 1 M Tris buffer, pH 9. The highly purified protein is subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity of the sequence is assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation and other analytical procedures as desired or necessary.

PRO175, PRO183, PRO198, PRO200, PRO202, PRO356, PRO364, PRO535, PRO819, PRO861, PRO877, PRO879, PRO882, PRO1002, PRO1304 and PRO1308 were expressed in high 5 cells by the above described method.

EXAMPLE 30

Preparation of Antibodies that Bind PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710

This Example illustrates preparation of monoclonal antibodies that can specifically bind PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710.

Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include purified PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 fusion proteins containing PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710, and cells expressing the gene encoding PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819,

PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

Mice, such as Balb/c, are immunized with the PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1 to 100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti-PRO175, anti-PRO200, anti-PRO198, anti-PRO364, anti-PRO356, anti-PRO535, anti-PRO819, anti-PRO1002; anti-PRO1308, anti-PRO1304, anti-PRO183, anti-PRO202, anti-PRO861, anti-PRO877, anti-PRO879, anti-PRO882 or anti-PRO1710 antibodies.

After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions generate hybridoma cells that can then be plated in 96-well tissue culture plates containing HAT medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells will be screened in an ELISA for reactivity against PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 is within the skill in the art.

The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-PRO175, anti-PRO200, anti-PRO198, anti-PRO364, anti-PRO356, anti-PRO535, anti-PRO819, anti-PRO1002, anti-PRO1308, anti-PRO1304, anti-PRO183, anti-PRO202, anti-PRO861, anti-PRO877, anti-PRO879, anti-PRO882 or anti-PRO1710 monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue-culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium-sulfate precipitation, followed by gel-exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

Deposit of Material

The following material(s) has/have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC):

<u>Material</u>	<u>ATCC Dep. No.</u>	<u>Deposit Date</u>
DNA19355-1150-1	209466	November 18, 1997

	DNA29101-1122	209653	March 5, 1998
	DNA33457-1078	209283	September 18, 1997
	DNA47365-1206	209436	November 7, 1997
	DNA47470-1130-P1	209422	October 28, 1997
5	DNA49143-1429	203013	June 23, 1998
	DNA57695-1340	203006	June 23, 1998
	DNA59208-1373	209881	May 20, 1998
	DNA62306-1570	203254	September 9, 1998
	DNA65406-1567	203219	September 15, 1998

- 10 This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc., and ATCC, which assures permanent and unrestricted availability of the progeny of the culture
- 15 of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886.0G 638).

- 20 The assignee of the present application has agreed that if a culture of the material(s) on deposit should die or be lost or destroyed when cultivated under suitable conditions, the material(s) will be promptly replaced on notification with another of the same. Availability of the deposited material(s) is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

- 25 The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct(s) deposited, since the deposited embodiment(s) is/are intended as single illustration(s) of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material(s) herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims
- 30 to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A composition comprising a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide or agonist or antagonist thereof, in admixture with a pharmaceutically acceptable carrier.
2. The composition of Claim 1 comprising a therapeutically effective amount of said polypeptide or said agonist or antagonist thereof.
3. The composition of Claim 1, wherein the agonist is an anti-PRO175, anti-PRO200, anti-PRO198, anti-PRO364, anti-PRO356, anti-PRO535, anti-PRO819, anti-PRO1002, anti-PRO1308, anti-PRO1304, anti-PRO183, anti-PRO202, anti-PRO861, anti-PRO877, anti-PRO879, anti-PRO882 or anti-PRO1710 antibody.
4. The composition of Claim 1, wherein the antagonist is an anti-PRO175, anti-PRO200, anti-PRO198, anti-PRO364, anti-PRO356, anti-PRO535, anti-PRO819, anti-PRO1002, anti-PRO1308, anti-PRO1304, anti-PRO183, anti-PRO202, anti-PRO861, anti-PRO877, anti-PRO879, anti-PRO882 or anti-PRO1710 antibody.
5. The composition of Claim 1 further comprising a cardiovascular, endothelial, angiogenic or angiostatic agent.
6. A method of preparing the composition of Claim 1 comprising admixing a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide or agonist or antagonist thereof, with a pharmaceutically acceptable carrier.
7. An article of manufacture comprising:
 - (1) a composition comprising (a) a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide, (b) an agonist of a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide, or (c) an antagonist of a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide, in admixture with a pharmaceutically acceptable carrier;
 - (2) a container containing said composition; and
 - (3) a label affixed to said container, or a package insert included in said container, referring to the use of said composition, in the treatment of a cardiovascular, endothelial, and angiogenic disorder.
8. The article of manufacture of Claim 7, wherein said agonist is an anti-PRO175, anti-PRO200, anti-PRO198, anti-PRO364, anti-PRO356, anti-PRO535, anti-PRO819, anti-PRO1002, anti-PRO1308, anti-PRO1304,

anti-PRO183, anti-PRO202, anti-PRO861, anti-PRO877, anti-PRO879, anti-PRO882 or anti-PRO1710 antibody.

9. The article of manufacture of Claim 7, wherein said antagonist is an anti-PRO175, anti-PRO200, anti-PRO198, anti-PRO364, anti-PRO356, anti-PRO535, anti-PRO819, anti-PRO1002, anti-PRO1308, anti-PRO1304, anti-PRO183, anti-PRO202, anti-PRO861, anti-PRO877, anti-PRO879, anti-PRO882 or anti-PRO1710 antibody.

10. The article of manufacture of Claim 7, wherein said composition comprises a therapeutically effective amount of said polypeptide or agonist or antagonist thereof, in admixture with said pharmaceutically acceptable carrier.

11. A method for identifying an agonist of a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide comprising:

(a) contacting cells and a test compound to be screened under conditions suitable for the induction of a cellular response normally induced by a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide; and

(b) determining the induction of said cellular response to determine if the test compound is an effective agonist, wherein the induction of said cellular response is indicative of said test compound being an effective agonist.

12. The method of Claim 11, wherein the cellular response normally induced by said polypeptide is stimulation of cell proliferation.

13. A method for identifying a compound that inhibits an activity of a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide comprising contacting a test compound with said polypeptide under conditions and for a time sufficient to allow the test compound and polypeptide to interact and determining whether the activity of said polypeptide is inhibited.

14. A method for identifying a compound that inhibits an activity of a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide comprising the steps of:

(a) contacting cells and a test compound to be screened in the presence of said polypeptide under conditions suitable for the induction of a cellular response normally induced by said polypeptide; and

(b) determining the induction of said cellular response to determine if the test compound is an effective antagonist.

15. The method of Claim 14, wherein the cellular response normally induced by said polypeptide is stimulation of cell proliferation.

16. A method for identifying a compound that inhibits the expression of a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide in cells that normally expresses the polypeptide, wherein the method comprises contacting the cells with a test compound under conditions suitable for allowing expression of said polypeptide and determining whether the expression of said polypeptide is inhibited.

17. An agonist of a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide.

18. An antagonist of a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide.

19. A compound that inhibits the expression of a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide in a mammalian cell which expresses said polypeptide.

20. The compound of Claim 19, wherein said compound is an antisense oligonucleotide.

21. An isolated antibody that binds to a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide.

22. The antibody of Claim 21 which is a monoclonal antibody.

23. The antibody of Claim 21 which is an antibody fragment.

24. The antibody of Claim 21 which is a single-chain antibody.

25. A method for diagnosing a disease or susceptibility to a disease which is related to a mutation in a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide-encoding nucleic acid sequence comprising determining the presence or absence of said mutation in said polypeptide-encoding nucleic acid sequence, wherein the presence or absence of said mutation is indicative of the presence of said disease or susceptibility to said disease.

26. A method of diagnosing a cardiovascular, endothelial or angiogenic disorder in a mammal which comprises analyzing the level of expression of a gene encoding a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide (a) in a test sample of tissue cells obtained from said mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower expression level in the test sample as compared to the control sample is indicative of the presence of a cardiovascular, endothelial or angiogenic disorder in said mammal.

27. A method of diagnosing a cardiovascular, endothelial or angiogenic disorder in a mammal which comprises detecting the presence or absence of a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide in a test sample of tissue cells obtained from said mammal, wherein the presence or absence of said polypeptide in said test sample is indicative of the presence of a cardiovascular, endothelial or angiogenic disorder in said mammal.

28. A method of diagnosing a cardiovascular, endothelial or angiogenic disorder in a mammal comprising (a) contacting an anti-PRO175, anti-PRO200, anti-PRO198, anti-PRO364, anti-PRO356, anti-PRO535, anti-PRO819, anti-PRO1002, anti-PRO1308, anti-PRO1304, anti-PRO183, anti-PRO202, anti-PRO861, anti-PRO877, anti-PRO879, anti-PRO882 or anti-PRO1710 antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between said antibody and a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide in the test sample, wherein the formation of said complex is indicative of the presence of a cardiovascular, endothelial or angiogenic disorder in the mammal.

29. A method for determining the presence of a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide in a sample comprising contacting a sample suspected of containing said polypeptide with an anti-PRO175, anti-PRO200, anti-PRO198, anti-PRO364, anti-PRO356, anti-PRO535, anti-PRO819, anti-PRO1002, anti-PRO1308, anti-PRO1304, anti-PRO183, anti-PRO202, anti-PRO861, anti-PRO877, anti-PRO879, anti-PRO882 or anti-PRO1710 antibody and determining binding of said antibody to a component of said sample.

30. A cardiovascular, endothelial or angiogenic disorder diagnostic kit comprising an anti-PRO175, anti-PRO200, anti-PRO198, anti-PRO364, anti-PRO356, anti-PRO535, anti-PRO819, anti-PRO1002, anti-PRO1308, anti-PRO1304, anti-PRO183, anti-PRO202, anti-PRO861, anti-PRO877, anti-PRO879, anti-PRO882 or anti-PRO1710 antibody and a carrier in suitable packaging.

31. A method for treating a cardiovascular, endothelial or angiogenic disorder in a mammal comprising administering to the mammal a therapeutically effective amount of a PRO175, PRO200, PRO198, PRO364,

PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide or agonist or antagonist thereof.

32. The method according to Claim 31, wherein the mammal is human.
33. The method of Claim 32, wherein the human has suffered myocardial infarction.
34. The method of Claim 32, wherein the human has cardiac hypertrophy, trauma, a cancer, or age-related macular degeneration.
35. The method of Claim 34, wherein the cardiac hypertrophy is characterized by the presence of an elevated level of $\text{PGF}_{2\alpha}$.
36. The method of Claim 31, wherein the PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide is administered together with a cardiovascular, endothelial or angiogenic agent.
37. The method of Claim 34, wherein the PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide is administered following primary angioplasty.
38. The method of Claim 31, wherein the cardiovascular, endothelial or angiogenic disorder is cancer.
39. The method of Claim 38, wherein the PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide is administered in combination with a chemotherapeutic agent, a growth inhibitory agent or a cytotoxic agent.
40. The method of Claim 31 wherein said agonist is an anti-PRO175, anti-PRO200, anti-PRO198, anti-PRO364, anti-PRO356, anti-PRO535, anti-PRO819, anti-PRO1002, anti-PRO1308, anti-PRO1304, anti-PRO183, anti-PRO202, anti-PRO861, anti-PRO877, anti-PRO879, anti-PRO882 or anti-PRO1710 antibody.
41. The method of Claim 31 wherein said antagonist is an anti-PRO175, anti-PRO200, anti-PRO198, anti-PRO364, anti-PRO356, anti-PRO535, anti-PRO819, anti-PRO1002, anti-PRO1308, anti-PRO1304, anti-PRO183, anti-PRO202, anti-PRO861, anti-PRO877, anti-PRO879, anti-PRO882 or anti-PRO1710 antibody.
42. A method for treating a cardiovascular, endothelial or angiogenic disorder in a mammal comprising administering to the mammal a nucleic acid molecule that encodes a PRO175, PRO200, PRO198, PRO364,

PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide or agonist or antagonist thereof.

43. The method of Claim 42 wherein said agonist is an anti-PRO175, anti-PRO200, anti-PRO198, anti-PRO364, anti-PRO356, anti-PRO535, anti-PRO819, anti-PRO1002, anti-PRO1308, anti-PRO1304, anti-PRO183, anti-PRO202, anti-PRO861, anti-PRO877, anti-PRO879, anti-PRO882 or anti-PRO1710 antibody.

44. The method of Claim 42 wherein said antagonist is an anti-PRO175, anti-PRO200, anti-PRO198, anti-PRO364, anti-PRO356, anti-PRO535, anti-PRO819, anti-PRO1002, anti-PRO1308, anti-PRO1304, anti-PRO183, anti-PRO202, anti-PRO861, anti-PRO877, anti-PRO879, anti-PRO882 or anti-PRO1710 antibody.

45. The method of Claim 42, wherein the mammal is human.

46. The method of Claim 42, wherein the nucleic acid molecule is administered via *ex vivo* gene therapy.

47. A recombinant retroviral particle comprising a retroviral vector consisting essentially of (1) a promoter, (2) nucleic acid encoding a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide or agonist or antagonist thereof, and (3) a signal sequence for cellular secretion of the polypeptide, wherein the retroviral vector is in association with retroviral structural proteins.

48. An *ex vivo* producer cell comprising a nucleic acid construct that expresses retroviral structural proteins and also comprises a retroviral vector consisting essentially of a (1) promoter, (2) nucleic acid encoding a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide or agonist or antagonist thereof, and (3) a signal sequence for cellular secretion of the polypeptide, wherein said producer cell packages the retroviral vector in association with the structural proteins to produce recombinant retroviral particles.

49. A method for inhibiting endothelial cell growth in a mammal comprising administering to the mammal a PRO175, PRO183, PRO198, PRO202, PRO364, PRO535, PRO819, PRO877, PRO879, PRO1002 or PRO1304 polypeptide or agonist thereof, wherein endothelial cell growth in said mammal is inhibited.

50. A method for stimulating endothelial cell growth in a mammal comprising administering to the mammal a PRO356, PRO861, PRO1308 or PRO1710 polypeptide or agonist thereof, wherein endothelial cell growth in said mammal is stimulated.

51. A method of inhibiting endothelial cell growth in a mammal comprising administering to the mammal an antagonist of a PRO356, PRO861, PRO1308 or PRO1710 polypeptide, wherein endothelial cell growth in said

mammal is inhibited.

52. A method of stimulating endothelial cell growth in a mammal comprising administering to the mammal an antagonist of a PRO175, PRO183, PRO198, PRO202, PRO364, PRO535, PRO819, PRO877, PRO879, PRO1002 or PRO1304 polypeptide, wherein endothelial cell growth in said mammal is stimulated.

53. A method for inducing cardiac hypertrophy in a mammal comprising administering to the mammal a PRO183, PRO200, PRO202 or PRO882 polypeptide or agonist thereof, wherein cardiac hypertrophy in said mammal is induced.

54. A method of reducing cardiac hypertrophy in a mammal comprising administering to the mammal an antagonist of a PRO183, PRO200, PRO202 or PRO882 polypeptide, wherein cardiac hypertrophy in said mammal is reduced.

55. A method for inhibiting angiogenesis induced by a PRO861 polypeptide in a mammal comprising administering a therapeutically effective amount of an anti-PRO861 antibody to the mammal, wherein said angiogenesis is inhibited.

56. A method for stimulating angiogenesis induced by a PRO861 polypeptide in a mammal comprising administering a therapeutically effective amount of said polypeptide to the mammal, wherein said angiogenesis is stimulated.

57. A method for inhibiting angiogenesis in a mammal comprising administering a therapeutically effective amount of a PRO198, PRO877, PRO1002 or PRO1304 polypeptide to the mammal, wherein said angiogenesis is inhibited.

58. A method for stimulating angiogenesis in a mammal comprising administering a therapeutically effective amount of an anti-PRO198, anti-PRO877, anti-PRO1002 or anti-PRO1304 polypeptide to the mammal, wherein said angiogenesis is stimulated.

59. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence that encodes an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:6), Figure 8 (SEQ ID NO:8), Figure 10 (SEQ ID NO:10), Figure 12 (SEQ ID NO:12), Figure 14 (SEQ ID NO:14), Figure 16 (SEQ ID NO:16), Figure 18 (SEQ ID NO:18), Figure 20 (SEQ ID NO:20), Figure 22 (SEQ ID NO:22), Figure 24 (SEQ ID NO:24), Figure 26 (SEQ ID NO:26), Figure 28 (SEQ ID NO:28), Figure 30 (SEQ ID NO:30), Figure 32 (SEQ ID NO:32), and Figure 34 (SEQ ID NO:34).

60. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence selected from the group consisting of the nucleotide sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:3), Figure 5 (SEQ ID NO:5), Figure 7 (SEQ ID NO:7), Figure 9 (SEQ ID NO:9), Figure 11 (SEQ ID NO:11), Figure 13 (SEQ ID NO:13), Figure 15 (SEQ ID NO:15), Figure 17 (SEQ ID NO:17), Figure 19 (SEQ ID NO:19), Figure 21 (SEQ ID NO:21), Figure 23 (SEQ ID NO:23), Figure 25 (SEQ ID NO:25), Figure 27 (SEQ ID NO:27), Figure 29 (SEQ ID NO:29), Figure 31 (SEQ ID NO:31), and Figure 33 (SEQ ID NO:33).

61. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence selected from the group consisting of the full-length coding sequence of the nucleotide sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:3), Figure 5 (SEQ ID NO:5), Figure 7 (SEQ ID NO:7), Figure 9 (SEQ ID NO:9), Figure 11 (SEQ ID NO:11), Figure 13 (SEQ ID NO:13), Figure 15 (SEQ ID NO:15), Figure 17 (SEQ ID NO:17), Figure 19 (SEQ ID NO:19), Figure 21 (SEQ ID NO:21), Figure 23 (SEQ ID NO:23), Figure 25 (SEQ ID NO:25), Figure 27 (SEQ ID NO:27), Figure 29 (SEQ ID NO:29), Figure 31 (SEQ ID NO:31), and Figure 33 (SEQ ID NO:33).

62. Isolated nucleic acid having at least 80% nucleic acid sequence identity to the full-length coding sequence of the DNA deposited under ATCC accession number 209466, 209653, 209283, 209436, 209422, 203013, 203006, 209881, 203254, or 203219.

63. A vector comprising the nucleic acid of any one of Claims 59 to 62.

64. The vector of Claim 63 operably linked to control sequences recognized by a host cell transformed with the vector.

65. A host cell comprising the vector of Claim 63.

66. The host cell of Claim 65, wherein said cell is a CHO cell.

67. The host cell of Claim 65, wherein said cell is an *E. coli*.

68. The host cell of Claim 65, wherein said cell is a yeast cell.

69. The host cell of Claim 65, wherein said cell is a Baculovirus infected insect cell.

70. A process for producing a PRO175, PRO200, PRO198, PRO364, PRO356, PRO535, PRO819, PRO1002, PRO1308, PRO1304, PRO183, PRO202, PRO861, PRO877, PRO879, PRO882 or PRO1710 polypeptide comprising culturing the host cell of Claim 65 under conditions suitable for expression of said polypeptide and recovering said polypeptide from the cell culture.

71. An isolated polypeptide having at least 80% amino acid sequence identity to an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:6), Figure 8 (SEQ ID NO:8), Figure 10 (SEQ ID NO:10), Figure 12 (SEQ ID NO:12), Figure 14 (SEQ ID NO:14), Figure 16 (SEQ ID NO:16), Figure 18 (SEQ ID NO:18), Figure 20 (SEQ ID NO:20), Figure 22 (SEQ ID NO:22), Figure 24 (SEQ ID NO:24), Figure 26 (SEQ ID NO:26), Figure 28 (SEQ ID NO:28), Figure 30 (SEQ ID NO:30), Figure 32 (SEQ ID NO:32), and Figure 34 (SEQ ID NO:34).

72. An isolated polypeptide scoring at least 80% positives when compared to an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:6), Figure 8 (SEQ ID NO:8), Figure 10 (SEQ ID NO:10), Figure 12 (SEQ ID NO:12), Figure 14 (SEQ ID NO:14), Figure 16 (SEQ ID NO:16), Figure 18 (SEQ ID NO:18), Figure 20 (SEQ ID NO:20), Figure 22 (SEQ ID NO:22), Figure 24 (SEQ ID NO:24), Figure 26 (SEQ ID NO:26), Figure 28 (SEQ ID NO:28), Figure 30 (SEQ ID NO:30), Figure 32 (SEQ ID NO:32), and Figure 34 (SEQ ID NO:34).

73. An isolated polypeptide having at least 80% amino acid sequence identity to an amino acid sequence encoded by the full-length coding sequence of the DNA deposited under ATCC accession number 209466, 209653, 209283, 209436, 209422, 203013, 203006, 209881, 203254, or 203219.

74. A chimeric molecule comprising a polypeptide according to any one of Claims 71 to 73 fused to a heterologous amino acid sequence.

75. The chimeric molecule of Claim 74, wherein said heterologous amino acid sequence is an epitope tag sequence.

76. The chimeric molecule of Claim 74, wherein said heterologous amino acid sequence is a Fc region of an immunoglobulin.

77. An antibody which specifically binds to a polypeptide according to any one of Claims 71 to 73.

78. The antibody of Claim 77, wherein said antibody is a monoclonal antibody, a humanized antibody or a single-chain antibody.

79. Isolated nucleic acid having at least 80% nucleic acid sequence identity to:

(a) a nucleotide sequence encoding the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:6), Figure 8 (SEQ ID NO:8), Figure 10 (SEQ ID NO:10), Figure 12 (SEQ ID NO:12), Figure 14 (SEQ ID NO:14), Figure 16 (SEQ ID NO:16), Figure 18 (SEQ ID NO:18), Figure 20 (SEQ ID NO:20), Figure 22 (SEQ ID NO:22), Figure 24 (SEQ ID NO:24), Figure 26 (SEQ ID NO:26), Figure 28 (SEQ ID NO:28), Figure 30 (SEQ ID NO:30), Figure 32 (SEQ ID NO:32), or Figure 34 (SEQ ID NO:34), lacking its

associated signal peptide;

(b) a nucleotide sequence encoding an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:6), Figure 8 (SEQ ID NO:8), Figure 10 (SEQ ID NO:10), Figure 12 (SEQ ID NO:12), Figure 14 (SEQ ID NO:14), Figure 16 (SEQ ID NO:16), Figure 18 (SEQ ID NO:18), Figure 20 (SEQ ID NO:20), Figure 22 (SEQ ID NO:22), Figure 24 (SEQ ID NO:24), Figure 26 (SEQ ID NO:26), Figure 28 (SEQ ID NO:28), Figure 30 (SEQ ID NO:30), Figure 32 (SEQ ID NO:32), or Figure 34 (SEQ ID NO:34), with its associated signal peptide; or

(c) a nucleotide sequence encoding an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:6), Figure 8 (SEQ ID NO:8), Figure 10 (SEQ ID NO:10), Figure 12 (SEQ ID NO:12), Figure 14 (SEQ ID NO:14), Figure 16 (SEQ ID NO:16), Figure 18 (SEQ ID NO:18), Figure 20 (SEQ ID NO:20), Figure 22 (SEQ ID NO:22), Figure 24 (SEQ ID NO:24), Figure 26 (SEQ ID NO:26), Figure 28 (SEQ ID NO:28), Figure 30 (SEQ ID NO:30), Figure 32 (SEQ ID NO:32), or Figure 34 (SEQ ID NO:34), lacking its associated signal peptide.

80. An isolated polypeptide having at least 80% amino acid sequence identity to:

(a) the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:6), Figure 8 (SEQ ID NO:8), Figure 10 (SEQ ID NO:10), Figure 12 (SEQ ID NO:12), Figure 14 (SEQ ID NO:14), Figure 16 (SEQ ID NO:16), Figure 18 (SEQ ID NO:18), Figure 20 (SEQ ID NO:20), Figure 22 (SEQ ID NO:22), Figure 24 (SEQ ID NO:24), Figure 26 (SEQ ID NO:26), Figure 28 (SEQ ID NO:28), Figure 30 (SEQ ID NO:30), Figure 32 (SEQ ID NO:32), or Figure 34 (SEQ ID NO:34), lacking its associated signal peptide;

(b) an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:6), Figure 8 (SEQ ID NO:8), Figure 10 (SEQ ID NO:10), Figure 12 (SEQ ID NO:12), Figure 14 (SEQ ID NO:14), Figure 16 (SEQ ID NO:16), Figure 18 (SEQ ID NO:18), Figure 20 (SEQ ID NO:20), Figure 22 (SEQ ID NO:22), Figure 24 (SEQ ID NO:24), Figure 26 (SEQ ID NO:26), Figure 28 (SEQ ID NO:28), Figure 30 (SEQ ID NO:30), Figure 32 (SEQ ID NO:32), or Figure 34 (SEQ ID NO:34), with its associated signal peptide; or

(c) an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:6), Figure 8 (SEQ ID NO:8), Figure 10 (SEQ ID NO:10), Figure 12 (SEQ ID NO:12), Figure 14 (SEQ ID NO:14), Figure 16 (SEQ ID NO:16), Figure 18 (SEQ ID NO:18), Figure 20 (SEQ ID NO:20), Figure 22 (SEQ ID NO:22), Figure 24 (SEQ ID NO:24), Figure 26 (SEQ ID NO:26), Figure 28 (SEQ ID NO:28), Figure 30 (SEQ ID NO:30), Figure 32 (SEQ ID NO:32), or Figure 34 (SEQ ID NO:34), lacking its associated signal peptide.

FIGURE 1

CAGCTCTCATTCTCCAAAAATGTTGTTTGGAGCCACTTGGAAAAATATGCCTTTAAGCCATTCAAGAACTCAA
GGAGCTCAGAGATCATCCTGGAAGCTGTGGCTCTTTTGCTCAATAGTTATGTTGCTATTTCTTTGCTCCTT
CAGTTGGCTAATCTTTATTTTTCTCCAATTAGAGACTGCTAAGGAGCCCTGTATGGCTAAGTTTGGACCAT
TACCCTCAAAATGGCAATGGCATCTTCTGAACCTCCTTGCCTGAATAAGGTGTCTGACTGGAAGCTGGAG
ATACCTCAGAATGGCTTATATTTAATTTATGGCCAAGTGGCTCCCAATGCAAACTACAATGATGTAGCTCC
TTTTGAGGTGCGGCTGTATAAAAAACAAAGACATGATACAACTCTAACAACAAATCTAAAATCCAAAATG
TAGGAGGGACTTATGAATTGCATGTTGGGGACACCATAGACTTGATATCAACTCTGAGCATCAGGTTCTAAAA
AATAATACATACTGGGGTATCATTTTACTAGCAAATCCCCAATTCATCTCCTAGAGACTTGATTTGATCTC
CTCATTCCTTCAGCACATGTAGAGGTGCCAGTGGGTGGATTGGAGGGAGAAGATATTCAATTTCTAGAGT
TTGTCTGTCTACAAAAATCAACACAAACAGAACTCCTCTGCACGTGAATTTTCATCTATCATGCCTATCTG
AAAGAGACTCAGGGGAAGAGCCAAAGACTTTTGGTTGGATCTGCAGAAATACTTCATTAATCCATGATAAA
ACAAATATGGATGACAGAGGACATGTGCTTTTCAAAGAATCTTTATCTAATTCTTGAATTCATGAGTGGAA
AAATGGAGTTCTATTCCCATGGAAGATTTACCTGGTATGCAAAAAGGATCTGGGGCAGTAGCCTGGCTTTG
TTCTCATATTCTTGGGCTGCTGTAATTCATTCTTCTCATACTCCCATCTTCTGAGACCCCTCCCAATAAAAA
GTAGACTGATAGGATGGCCACAGATATGCCTACCATACCCTACTTTAGATATGGTGGTGTAGAAAGATAAA
GAACAATCTGAGAACTATTGGAATAGAGGTACAAGTGGCATAAAATGGAATGTACGCTATCTGAAAATTTT
TCTTGGTTTTATCTTCTCAGGATGCAGGGTGTCTTTAAAAAGCCTTATCAAAGGAGTCATCCGAACCTC
ACGTAGAGCTTTGTGAGACCTTACTGTTGGTGTGTGTGTCTAAACATTGCTAATTGTAAAGAAAGAGTAAC
CATTAGTAATCATTAGGTTTTAACCCAGAATGGTATTATCATTACTGGATTATGTCATGTAATGATTTAGT
ATTTTGTAGCTAGCTTTCCACAGTTTGCAGAGTGCTTTTCGTAAAACAGTTAGCAATTCATGAAGTTAATTG
GGCAGGCATTTGGGGGAAAATTTTAGTGATGAGAATGTGATAGCATAGCATAGCCAACCTTCTCAACTCA
TAGGACAAGTGACTACAAGAGGCAATGGGTAGTCCCCTGCATTGCACTGTCTCAGCTTTAGAATTGTTATT
TCTGCTATCGTGTTATAAGACTCTAAAACCTAGCGAATTCACTTTTCAGGAAGCATATTTCCCTTTAGCCC
AAGGTGAGCAGAGTGAAGCTACAACAGATCTTTCTTTACCAGCACACTTTTTTTTTTTTTTCTGCTGA
ATCAGGGAGATCCAGGATGCTGTTCAAGCCAAATCCCAACCAATTTCCCTTTTCACTTTGCAGGGCCCAT
CTTAGTCAAATGTGCTAACTTCTAAAATAATAAATAGCACTAATTCAAAATTTTTGGAACTTTAAATTAGC
TACTTGCNGGTTGCTTGTGAAAGGNATATAATGATTACATTGTAAACAAATTTAAATATTTATGGATAT
TTGTGAAAAGCTGCATTATGTTAAATAATATTACATGTAAAGCT

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FIGURE 2

MCLSHLENMPLSHSRTQGAQRSSWKLWLFCSIVMLLFLCSFSWLIFIFLQLETAKPEPCMAKFGPLPSKWQM
ASSEPPCVNKKVSDWKLEILQNGLYLIYGQVAPNANYNDVAPFEVRLYKNKDMIQTLTNKSKIQNVGGTYEL
HVGDTIDLIFNSEHQVLKNNTYWGIIILLANPQFIS

Signal peptide:	Amino acids 1-25
Transmembrane Domain:	Amino acids 26-51
Extracellular Domain:	Amino acids 52-177
N-linked glycosylation sites:	Amino acids 129-133;161-165
N-myristoylation site:	Amino acids 18-24

FIGURE 3

CGGACGCGTGGGCGGACGCGTGGGCGGACGCGTGGGCGGACGCGTGGGCTGGTTCAGGTCCAGGTTTTGTCT
TTGATCCTTTTCAAAAACCTGGAGACACAGAAGAGGGCTCTAGGAAAAAGTTTTGGATGGGATTATGTGGAA
ACTACCTGCGATTCTCTGCTGCCAGAGCAGGCTCGGCGCTTCCACCCAGTGAGCCTTCCCTGGCGGT
GGTGAAGAGAGACTCGGGAGTCGCTGCTTCCAAAGTGCCCGCGGTGAGTGAGCTCTACCCAGTCAGCCAA
ATGAGCCTCTTCGGGCTTCTCCTGCTGACATCTGCCCTGGCCGGCCAGAGACAGGGGACTCAGGCGGAATC
CAACCTGAGTAGTAAATCCAGTTTTCCAGCAACAAGGAACAGAACGGAGTACAAGATCCTCAGCATGAGA
GAATTTACTGTGTCTACTAATGGAAGTATTCACAGGCCAAGGTTTTCTCATACTTATCCAGAAATACG
GTCTTGGTATGGAGATTAGTAGCAGTAGAGGAAAATGTATGGATACAACCTTACGTTTGTGAAAGATTTGG
GCTTGAAGACCCAGAAGATGACATATGCAAGTATGATTTTGTAGAAGTTGAGGAACCCAGTGATGGAACATA
TATTAGGGCGCTGGTGTGGTTCTGGTACTGTACCAGGAAAAAGATTTCTAAAGGAAATCAAATTAGGATA
AGATTTGTATCTGATGAATATTTTCTTCTGAACCAGGGTTCTGCATCCACTACAACATTGTCTATGCCACA
ATTCACAGAAGCTGTGAGTCCTTCAGTGCTACCCCTTCAGCTTTGCCACTGGACCTGCTTAATAATGCTA
TAACTGCCTTTAGTACCTTGAAGACCTTATTGATATCTTGAACCAGAGAGATGGCAGTTGGACTTAGAA
GATCTATATAGGCCAATTTGGCAACTTCTTGGCAAGGCTTTTGTTTTTTGAAGAAAAATCCAGAGTGGTGA
TCTGAACCTTCAACAGAGGAGGTAAGATTATACAGCTGCACACCTCGTAACTTCTCAGTGTCTATGAAGG
AAGAACTAAAGAGAACCAGTACCAATTTCTGGCCAGGTTGTCTCTGGTTAAACGCTGTGGTGGGAAGTGT
GCCTGTGTCTCCACAATTGCAATGAATGTCAATGTGTCCCAAGCAAAGTTACTAAAAAATACCAGAGGT
CCTTCAGTTGAGACCAAAGACCGGTGTGAGGGGATTGCACAAATCACTCACCGACGTGGCCCTGGAGCACC
ATGAGGAGTGTGACTGTGTGTGCAGAGGGAGCACAGGAGGATAGCCGCATCACCAACAGCAGCTCTTGCCC
AGAGCTGTGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGTTGT
TTGCTTCAAGGACCTTTTCTCTCAGGATTTACAGTGCATTCTGAAAGAGGAGACATCAAACAGAAATTAGG
AGTTGTGCAACAGCTCTTTTGAGAGGAGGCCTAAAGGACAGGAGAAAAAGGTCTTCAATCGTGGAAAGAAAA
TTAAATGTTGTATTAAATAGATCACCAGCTAGTTTACAGTTTACCATGTACGTATTTCCACTAGCTGGGTTCT
TGTATTTAGTTCTTTTCGATACGGCTTAGGGTAATGTGAGTACAGGAAAAAACTGTGCAAGTGAGCACCT
GATTCGGTTGCCTTGCTTAACTCTAAAGCTCCATGTCTGGGCCCTAAATCGTATAAAATCTGGATTTTTT
TTTTTTTTTTTGTCTCATATTCACATATGTAAACCAGAACATTTCTATGTACTACAAACCTGGTTTTTAAAAA
GGAATATGTTGCTATGAATTAACCTTGTGTCTGCTGATAGGACAGACTGGATTTTTCTATATTTCTTATT
AAAATTTCTGCCATTTAGAAGAAGAGAACTACATTCATGGTTTGGAAAGAGATAAACTGAAAAGAGAGTG
GCCTTATCTTCACTTTATCGATAAGTCAGTTTATTTGTTTCATTGTGTACATTTTTATATTCTCCTTTTGA
CATTATAACTGTTGGCTTTTCTAATCTTGTTAAATATATCTATTTTTTACCAAAGGTATTTAATATTCTTTT
TTATGACAACTTAGATCAACTATTTTAGCTTGGTAAATTTTTCTAAACACAATTGTTATAGCCAGAGGAA
CAAAGATGATATAAAATATTGTTGCTCTGACAAAAATACATGTATTTTCTTCTCGTATGGTGCTAGAGTTA
GATTAATCTGCATTTTAAAAAACTGAATTGGAATAGAATTGGTAAGTTGCAAAGACTTTTTGAAAAATAATT
AAATTATCATATCTTCCATTCTGTATTGGAGATGAAAAATAAAAGCAACTTATGAAAGTAGACATTGAG
ATCCAGCCATTACTAACCTATTCTTTTTTGGGGAAATCTGAGCCTAGCTCAGAAAAACATAAAGCACCTT
GAAAAAGACTTGGCAGCTTCTGATAAAGCGTGTCTGTGCTGTGAGTAGGAACACATCTATTATTGTGTA
TGTGTGGTTTTTATTATCTTAACTCTGTTCCATACACTTGTATAAATACATGGATATTTTTTATGTACAGA
AGTATGTCTCTTAACCAGTTCACTTATTGTACTCTGGCAATTTAAAGAAAAATCAGTAAATATTTTGCTT
GTAAATGCTTAATATNGTGCCTAGGTTATGTGGTGACTATTTGAATCAAAATGTATTGAATCATCAAAT
AAAAGAAATGTGGCTATTTTGGGGAGAAAATTAAGGAAAAAGGTTTAGGGATAACAGGGT
AATGCGGCC

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FIGURE 4

signal sequence:	Amino acids 1-14
N-glycosylation sites:	Amino acids 25-29;55-59;254-258
N-myristoylation sites:	Amino acids 15-21;117-123;127-133; 281-287;282-288;319-325
Amidation site:	Amino acids 229-233

MSLFGLLLLSALAGQRQGTQAESNLSSKFQFSSNKEQNGVQDPQHERIITVSTNGSIHSRFPHTYPRNT
VLVWRLVAVEENVWIQLTFDERFGLEDPEDDICKYDFVEVEEPSDGTILGRWCGSGTVPGKQISKGNQIRI
RFVSDEYFPSEPGFCIHYNIVMPQFTEAVSPSVLPSPALPLDLLNNAITAFSTLEDLIRYLEPERWQLDLE
DLYRPTWQLLGKAFVFGRKSRVVDLNLLEEVRLYSTPRNFSVSIREELKRTDTIFWPGCLLVKRCGGNC
ACCLHNCNECQCVPSKVTKKYHEVLQLRPKTGVRGLHKSLTDVALEHHEECDVCVRGSGTGG

FIGURE 5

CGGACGCGTGGGCCCCCTGGTGGGCCCAGCAAGATGGATCTACTGTGGATCCTGCCCTCCCTGTGGCTTCTC
CTGCTTGGGGGGCCTGCCTGCCTGAAGACCCAGGAACACCCAGCTGCCCAGGAACCCAGGGAACCTGGAAGC
CAGCAAAGTTGTCCTCCTGCCCAAGTTGTCCCGGAGCTCCAGGAAGTCCTGGGGAGAAGGGAGCCCCAGGTC
CTCAAGGGCCACCTGGACCACCAGGCAAGATGGGCCCCAAGGGTGAGCCAGGCCCCAGAACTGCCGGGAG
CTGTTGAGCCAGGGCGCCACCTTGAGCGGCTGGTACCATCTGTGCCTACCTGAGGGCAGGGCCCTCCCACT
CTTTTGTGACATGGACACCGAGGGGGCGGCTGGCTGGTGTTCAGAGGCGCCAGGATGGTTCTGTGGATT
TCTTCCGCTCTTGGTCCTCCTACAGAGCAGGTTTTGGGAACCAAGAGTCTGAATTCTGGCTGGGAAATGAG
AATTTGCACCAGCTTACTCTCCAGGGTAACTGGGAGCTGCGGGTAGAGCTGGAAGACTTTAATGGTAACCG
TACTTTCGCCCCTATGCGACCTTCCGCTCCTCGGTGAGGTAGACCACTACCAGCTGGCACTGGGCAAGT
TCTCAGAGGGCACTGCAGGGGATTCCCTGAGCCTCCACAGTGGGAGGCCCTTTACCACCTATGACGCTGAC
CACGATTCAAGCAACAGCAACTGTGCAGTGATTGTCCACGGTGCCTGGTGGTATGCATCCTGTTACCGATC
AAATCTCAATGGTCGCTATGCAGTGTCTGAGGCTGCCGCCACAAATATGGCATTGACTGGGCCTCAGGCC
GTGGTGTGGGCCACCCCTACCGCAGGGTTCCGATGATGCTTCGATAGGGCACTCTGGCAGCCAGTGCCTT
ATCTCTCCTGTACAGCTTCCGGATCGTCAGCCACCTTGCCCTTGCCAACCACCTCTGCTTGCCTGTCCACA
TTTAAAAATAAAATCATTTTAGCCCTTTCA

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FIGURE 6

MDLLWILPSLWLLLLGGPACLKTQEHPSCPGPRELEASKVLLPSCPGAPGSPGEGKAPGPQGPPGPPGKM
GPKGEPGPRNCRELLSQGATLSGWYHLCLPEGRALPVFCMDTEGGGWLVFQRRQDGSVDFFRSWSSYRAG
FGNQESEFWLGNENLHQLTLQGNWELRVELEDENGNRTFAHYATFRLLGEVDHYQLALGKFSEGTAGDSLS
LHSGRPFTTYDADHDSSNSNCAVIVHGAWWYASCYRSNLNGRYAVSEAAAHKYGIDWASGRGVGHPYRRVR
MMLR

Signal peptide:	Amino acids 1-16
N-glycosylation site:	Amino acids 178-182
Glycosaminoglycan attachment site:	Amino acids 272-276
Tyrosine kinase phosphorylation site:	Amino acids 188-197
N-myristoylation sites:	Amino acids 16-22;89-95;144-150; 267-273
Fibrinogen beta and gamma chains C-terminal domain signature:	Amino acids 242-255

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FIGURE 7

CACGCACTTCACCTGGGTCGGGATTCTCAGGTCATGAACGGTCCCAGCCACCTCCGGGCAGGGCGGGTGAG
GACGGGGACGGGGCGTGTCCAACCTGGCTGTGGGCTCTTGAAACCCGAGCATGGCACAGCACGGGGCGATGG
GCGCGTTTCGGGGCCCTGTGCGGCCTGGCGCTGCTGTGCGCGCTCAGCCTGGGTGAGCGCCCCACCGGGGT
CCCGGGTGCGGCCCTGGGCGCTCCTGCTTGGGACGGGAACGGACGCGCGCTGCTGCCGGGTTACACGAC
GCGCTGCTGCCGCGATTACCCGGGCGAGGAGTGCTGTTCCGAGTGGGACTGCATGTGTGTCCAGCCTGAAT
TCCACTGCGGAGACCCCTTGCTGCACGACCTGCCGGCACCACCCTTGTCCTCCCGAGGCCAGGGGGTACAGTCC
CAGGGGAAATTACAGTTTTGGCTTCCAGTGTATCGACTGTGCCTCGGGGACCTTCTCCGGGGGCCACGAAGG
CCACTGCAAACCTTGGACAGACTGCACCCAGTTCGGGTTTCTCACTGTGTTCCCTGGGAACAAGACCCACA
ACGCTGTGTGCGTCCCAGGGTCCCGCCGGCAGAGCCGCTTGGGTGGCTGACCGTCGTCTCCTGGCCGTG
GCCGCTGCGTCCTCCTCCTGACCTCGGCCCAGCTTGGACTGCACATCTGGCAGCTGAGGAGTCAGTGCA
GTGGCCCCGAGAGACCCAGCTGCTGCTGGAGGTGCCGCCGTCGACCGAAGACGCCAGAAGCTGCCAGTTCC
CCGAGGAAGAGCGGGGCGAGCGATCGGCAGAGGAGAAGGGCGGGCTGGGAGACCTGTGGGTGTGAGCCTGG
CCGTCTCCGGGGCCACCGACCGCAGCCAGCCCCCTCCCAGGAGCTCCCCAGGCCGCGAGGGGCTCTGCGTT
CTGCTCTGGGCCCGGGCCCTGCTCCCCTGGCAGCAGAAGTGGGTGCAGGAAGGTGGCAGTGACCAGCGCCCT
GGACCATGCAGTTC

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FIGURE 8

MAQHGMGAFRALCGLALLCALSLGQRPTGGPGCGPGRLLLGTGTDARCCRVHTTRCCRDYPGEECCSEWD
CMCVQPEFHCGDPCCTTCRHHPCPPGQGVQSQGFSGFGQCIDCASGTFSGGHEGHCKPWTDCQFGFLT
VFPNGKTHNAVCPGSPPAEPLGWLTVVLLAVAACVLLLTSAQLGLHIWQLRSQCMWPRETQLLEVPSTE
DARSCQFPPEERGERSAEEKGRLGDLWV

Signal sequence:	Amino acids 1-25
Potential transmembrane domain:	Amino acids 162-180
N-glycosylation site:	Amino acids 146-150
N-myristoylation sites:	Amino acids 5-11;8-14;25-31;30-36; 33-39;118-124;122-128;156-162
Prokaryotic membrane lipoprotein lipid attachment site:	Amino acids 166-177
Leucine zipper pattern:	Amino acids 171-193

FIGURE 9

GAAAGCTATAGGCTACCCATTTCAGCTCCCCTGTGAGAGACTCAAGCTTTGAGAAAGGCTAGCAAAGAGCAA
GGAAAGAGAGAAAAACAACAAAGTGGCGAGGCCCTCAGAGTGAAAGCGTAAGGTTTCAGTCAGCCTGCTGCAG
CTTTGTCAGACCTCAGCTGGGCATCTCCAGACTCCCCTGAAGGAAGAGCCTTCCTCACCCAAACCCACAAAAG
ATGCTGAAAAAGCCTCTCTCAGCTGTGACCTGGCTCTGCATTTTCATCGTGGCCTTTGTGAGCCACCCAGC
GTGGTGCAGAAAGCTCTCTAAGCACAAGACACCAGCACAGCCACAGCTCAAAGCGGCCAACTGCTGTGAGG
AGGTGAAGGAGCTCAAGGCCCAAGTTGCCAACCTTAGCAGCCTGCTGAGTGAAGTGAACAAGAAGCAGGAG
AGGGACTGGGTGAGCGTGGTCATGCAGGTGATGGAGCTGGAGAGCAACAGCAAGCGCATGGAGTCGCGGGCT
CACAGATGCTGAGAGCAAGTACTCCGAGATGAACAACCAAATTGACATCATGCAGCTGCAGGCAGCACAGA
CGGTCACTCAGACCTCCGCAGATGCCATCTACGACTGCTCTTCCCTCTACCAGAAGAACTACCGCATCTCT
GGAGTGATAAGCTTCTCTCTGATGACTTCTTGGGCAGCCCTGAACTGGAGGTGTTCTGTGACATGGAGAC
TTCAGGCCGAGGCTGGACCATCATCCAGAGACGAAAAAGTGGCCTTGCTCTCTTCTACCGGACTGGAAGC
AGTACAAGCAGGGCTTTGGCAGCATCCGTGGGACTTCTGGCTGGGGAACGAACACATCCACCGGCTCTCC
AGACAGCCAACCCGGCTGCGTGTAGAGATGGAGGACTGGGAGGGCAACCTGCGCTACGCTGAGTATAGCCA
CTTTGTTTGGGCAATGAACTCAACAGCTATCGCCTCTTCTGGGGAACACTGGAATGTGGGGAACG
ACGCCCTCCAGTATCATAACAACACAGCCTTCAGCACCAAGGACAAGGACAATGACAACCTGCTTGGACAAG
TGTGCACAGCTCCGCAAGGTGGCTACTGGTACAACCTGCTGCACAGACTCCAACCTCAATGGAGTGTACTA
CCGCCTGGGTGAGCACAATAAGCACCTGGATGGCATCACCTGGTATGGCTGGCATGGATCTACCTACTCCC
TCAAACGGGTGGAGATGAAAAATCCGCCCAGAAGACTTCAAGCCTTAAAGGAGGCTGCCGTGGAGCACGGA
TACAGAACTGAGACACGTGGAGACTGGATGAGGGCAGATGAGGACAGGAAGAGAGTGTAGAAAGGGTAG
GACTGAGAAACAGCCTATAATCTCCAAAGAAAGAATAAGTCTCCAAGGAGCACAAAAAATCATATGTACC
AAGGATGTTACAGTAAACAGGATGAAGTATTTAAACCCACTGGGTCTGCCACATCCTTCTCAAGGTGGTA
GACTGAGTGGGGTCTCTCTGCCCAAGATCCCTGACATAGCAGTAGCTTGTCTTTTCCACATGATTTGTCTG
TGAAAGAAAATAATTTTGGAGATCGTTTTATCTATTTTCTCTACGGCTTAGGCTATGTGAGGGCAAAACACA
AATCCCTTTTGCTAAAAAGAACCATATTATTTTGAATCTCAAAGGATAGGCCTTTGAGTGTAGAGAAAGGA
GTGAAGGAGGCAGGTGGGAAATGGTATTTCTATTTTAAATCCAGTGAAATTATCTTGAGTCTACACATTA
TTTTTAAACACAAAAATTGTTCCGCTGGAACTGACCCAGGCTGGACTTGCGGGGAGGAACTCCAGGGCA
CTGCATCTGGCGATCAGACTCTGAGCACTGCCCTGCTCGCCTTGGTCATGTACAGCACTGAAAGGAATGA
AGCACCAGCAGGAGGTGGACAGAGTCTCTCATGGATGCCGGCACAAAACCTGCCTTAAAAATTTTATAGTTA
ATACAGGTATATCTATTTTATTTTACTTTGTAAGAAACAAGCTCAAGGAGCTTCTTTTAAATTTTGTCTG
TAGGAAATGGTTGAAACTGAAGGTAGATGGTGTATAGTTAATAATAAATGCTGTAAATAAGCATCTCACTTT
GTAAAAATAAAATATTGTGGTTTTGTTTTAAACATTCAACGTTTCTTTCTCTTCTACAATAAACACTTTCA
AAATGTG

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FIGURE 10

MLKKPLSAVTWLCIFIVAFVSHPAWLQKLSKHKTPAQPOLKAANCCEEVKELKAQVANLSSLLSELNKKQE
RDWVSVMQVMELESNSKRMSRLTDAESKYSEMNNQIDIMQLQAAQTVTQTSADAIYDCSSLYQKNYRIS
GVYKLPPDDFLGSPLELVFCDMETSGGGWTIIQRRKSGLVSFYRDWKQYKQGFGSIRGDFWLGNHHRLS
RQPTRLRVEMEDWEGNLRVYAEYSHFVLGNELNSYRLFNGNYTGNVGNDAQYHNNTAFSTKDKDNDNCLDK
CAQLRKGGYWNCCTDSNLNGVYYRLGEHNKHLDGITWYGWHGSTYSLKRVEMKIRPEDFKP

N-glycosylation sites: Amino acids 58-62;253-257;267-271

Glycosaminoglycan attachment site: Amino acids 167-171

cAMP- and cGMP-dependent protein kinase phosphorylation site:
Amino acids 176-180

N-myristoylation sites: Amino acids 168-174;196-202;241-247;
252-258;256-262;327-333

Cell attachment sequence: Amino acids 199-202

Signal sequence: Amino acids 1-26

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FIGURE 11

CCGCGGGAACGCTGTCCTGGCTGCCGCCACCCGAACAGCCTGTCCTGGTGCCCCGGCTCCCTGCCCCGCGC
CCAGTCATGACCCCTGCGCCCCCTCACTCCTCCCGCTCCATCTGCTGCTGCTGCTGCTCAGTGCGGCGGT
GTGCCGGGCTGAGGCTGGGCTCGAAACCGAAAGTCCCGTCCGGACCCCTCCAAGTGGAGACCCTGGTGGAGC
CCCCAGAACCATGTGCCGAGCCCGCTGCTTTTGGAGACACGCTTCACATACACTACACGGGAAGCTTGGTA
GATGGACGTATTATTGACACCTCCCTGACCAGAGACCCCTCTGGTTATAGAAGCTTGGCCAAAAGCAGGTGAT
TCCAGGTCTGGAGCAGAGTCTTCTCGACATGTGTGTGGGAGAGAAGCGAAGGGCAATCATTCTTCTCACT
TGGCCTATGAAAAACGGGGATTTCCACCATCTGTCCAGCGGATGCAGTGGTGCAGTATGACGTGGAGCTG
ATTGCACTAATCCGAGCCAACCTACTGGCTAAAGCTGGTGAAGGGCATTTTGCCTCTGGTAGGGATGGCCAT
GGTGCCAGCCCTCCTGGGCCTCATTGGGTATCACCTATACAGAAAGGCCAATAGACCCAAAGTCTCCAAAA
AGAAGCTCAAGGAAGAGAAACGAAACAAGAGCAAAAAGAAATAATAAATAATAAATTTAAAAAACTTAA
AAAAAAAAAAAAAA

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FIGURE 12

MTLRPSLLPLHLLLLLLLLSAAVCRAEAGLETESPVRTLQVETLVEPPEPCAEPAAFGDTLHIHYTGSLVDG
RIIDTSLTRDPLVIELGQKQVIPGLEQSLDMCVGEKRRAIIPSHLAYGKRGFPPSPADAVVQYDVELIA
LIRANYWLKLVKGILPLVGMAMVPALLGLIGYHLYRKANRPKVSKKKLKEEKRNKSKKK

Signal peptide:	Amino acids 1-25
Transmembrane domain:	Amino acids 155-174
N-glycosylation site:	Amino acids 196-200
N-myristoylation site:	Amino acids 95-101
Amidation site:	Amino acids 119-123
FKBP-type peptidyl-prolyl cis-trans isomerase signature 1:	Amino acids 62-78;87-124;128-142

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FIGURE 13

CAGTTCTGAAATCAATGGAGTTAATTTAGGGAATACAAACCAGCCATGGGGGTGGAGATTGCCTTTGCCTC
AGTGATTCTCACCTGCCTCTCCCTTCTGGCAGCAGGAGTCTCCAGGTTGTTCTTCTCCAGCCAGTTCCAA
CTCAGGAGACAGGTCCCAAGGCCATGGGAGATCTCTCCTGTGGCTTTGCCGGCCACTCATGAGAGTGTTTT
TGTGTAAAGTATTTTTTAGAATACTGTTGACTTCTTCATGATTTAATAACCATCCTTTGCCGAAGTTTTATGA
GGCTTTAGGGGAATGTCAACCCTCAAATTTTTGTTATACTAGATGGCTTCCATTTACCCACCACTATTTTA
AGGTCCCTTTATTTTTAGGTTCAAGGTTCAATTTGACTTGAGAAAGTGCCCTTCTGCAGCTTCATTGATTTT
GTTTATCTTCACTATTAATTGTAACGATTAAAAAGAATAAGAGCACGCAGACCTCTAGGAGAATATTTTA
TCCCTGGGTGCCCCTGACACATTTATGTAGTGATCCCAAAATGTGATTGTTAATTTAAATGTTATTCTAA
TATTAGTACATTCAGTTGTGATGTAATATGAATAACCAGAATCTATTTCTTAAAAGTTTTGAGTATATTTT
TCAACTAGATATTTGTATAGAAAGACTGAATAGTGATG

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FIGURE 14

MGVEIAFASVILTCLSLAAGVSQVLLQPVPPTQETGPKAMGDLSCGFAGHS

Signal peptide: Amino acids 1-24

N-myristoylation site: Amino acids 2-8

Homologous region to Ig light chain: Amino acids 5-33

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FIGURE 15

TCTCAGACTCTTGGAAGGGGCTATACTAGACACACAAAGACAGCCCCAAGAAGGACGGTGGAGTAGTGTCC
TCGCTAAAAGACAGTAGATATGCAACGCCTCTTGCTCCTGCCCTTTCTCCTGCTGGGAACAGTTTCTGCTC
TTCATCTGGAGAATGATGCCCCCATCTGGAGAGCCTAGAGACACAGGCAGACCTAGGCCAGGATCTGGAT
AGTTCAAAGGAGCAGGAGAGAGACTTGGCTCTGACGGAGGAGGTGATTCAAGCAGAGGGAGAGGAGGTCAA
GGCTTCTGCCTGTCAAGACAACCTTTGAGGATGAGGAAGCCATGGAGTCGGACCCAGCTGCCTTAGACAAGG
ACTTCCAGTGCCCCAGGGAAGAAGACATTGTTGAAGTGCAGGGAAGTCCAAGGTGCAAGACCTGCCGCTAC
CTATTGGTGC GGACTCCTAAAACCTTTTGCAGAAGCTCAGAATGTCTGCAGCAGATGCTACGGAGGCAACCT
TGTCTCTATCCATGACTTCAACTTCAACTATCGCATTCAAGTGCTGCACTAGCACAGTCAACCAAGCCCAGG
TCTGGATTGGAGGCAACCTCAGGGGCTGGTTTCTGTGGAAGCGGTTTGTGCTGGACTGATGGGAGCCACTGG
AATTTTGCTTACTGGTCCCCAGGGCAACCTGGGAATGGGCAAGGCTCCTGTGTGGCCCTATGCACCAAAGG
AGGTTATTGGCGACGAGCTCAATGCGACAAGCAACTGCCCTTCGTCTGCTCCTTCTAAGCCAGCGGCACGG
AGACCCTGCCAGCAGCTCCCTCCCGTCCCCAACCTCTCCTGCTCATAAATCCAGACTTCCACAGCAAAA
AAAAAAAAAAAAA

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FIGURE 16

MQRLLLLPFLLLGTVSALHLENDAPHLESLETQADLGQDLDSKEQERDLALTEEVIAEGEEVKASACQD
NFEDEEAMESDPAALDKDFQCPREEDIVEVQGS PRCKTCRYLLVRTPKTFAEAQNVCSR CYGGNLVSIHDF
NFNRYIQCCTSTVNQAQVWIGGNLRGWFLWKRFCWTDGSHWNFAYWSPGQPGNGQGSCVALCTKGGYWRRRA
QCDKQLPFVCSF

Signal peptide:

Amino acids 1-17

N-myristoylation sites:

Amino acids 13-19;103-109;134-140;
164-170;180-186;191-197;194-200;
196-202;198-204

C-type lectin domain signature:

Amino acids 200-224

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FIGURE 17

CGTCTCTGCGTTTCGCCATGCGTCCCGGGGCGCCAGGGCCACTCTGGCCTCTGCCCTGGGGGGCCCTGGCTT
GGGCCGTGGGCTTCGTGAGCTCCATGGGCTCGGGGAACCCCGCGCCCGGTGGTGTTCGTGGCTCCAGCAG
GGCCAGGAGGCCACCTGCAGCCTGGTGCTCCAGACTGATGTACCCGGGCGGAGTGCTGTGCCTCCGGCAA
CATTGACACCGCTGGTCCAACCTCACCCACCCGGGGAACAAGATCAACCTCCTCGGCTTCTTGGGCCTTG
TCCACTGCCTTCCCTGCAAAGATTTCGTGCGACGGCGTGAGTGCGGCCCCGGGCAAGGCGTGCCGCATGCTG
GGGGGCGCCCGCGCTGCGAGTGCGCGCCCGACTGCTCGGGGCTCCCGGCGCGGCTGCAGGTCTGCGGCTC
AGACGGCGCCACCTACCGCGACGAGTGCGAGCTGCGCGCCGCGCGCTGCCGCGGCCACCCGGACCTGAGCG
TCATGTACCGGGGCGCTGCCGCAAGTCTGTGAGCAGTGGTGTGCCGCGGCCACAGTCGTGCGTCTGTG
GACCAGACGGGCGAGCGCCCACTGCGTGGTGTGTCGAGCGGCGCCCTGCCCTGTGCCCTCCAGCCCCGGCCA
GGAGCTTTGCGGCAACAACAACGTACCTACATCTCCTCGTGCCACATGCGCCAGGCCACCTGCTTCCTGG
GCCGCTCCATCGGCGTGCGCCACGCGGGCAGCTGCGCAGGCACCCCTGAGGAGCGGCCAGGTGGTGGTCT
GCAGAAGAGGAAGAGAACTTCGTGTGAGCCTGCAGGACAGGCCCTGGGCTGGTGCCCGAGGCCCCCCATCA
TCCCTGTATTATTATTGCCCACAGCAGAGTCTAATTATATGCCACGGACACTCCTTAGAGCCCCGATTCCG
ACCACTTGGGGATCCAGAACCTCCCTGACGATATCCTGGAAGGACTGAGGAAGGGAGGCTGGGGGCGCG
CTGGTGGGTGGGATAGACCTGCGTTCGCGACACTGAGCGCCTGATTTAGGGCCCTTCTCTAGGATGCCCA
GCCCCCTACCCTAAGACCTATTGCCGGGGAGGATTCCACACTTCCGCTCCTTTGGGGATAAACCTATTAAAT
ATTGCTACTATCAAGAGGGCTGGGCATTCTCTGCTGGTAATTCTGAAGAGGCATGACTGCTTTCTCAGC
CCCAAGCCTCTAGTCTGGGTGTGTACGGAGGGTCTAGCCTGGGTGTGTACGGAGGGTCTAGCCTGGGTGAG
TACGGAGGGTCTAGCCTGGGTGAGTACGGAGGGTCTAGCCTGGGTGAGTACGGAGGGTCTAGCCTGGGTG
GTATGGAGGATCTAGCCTGGGTGAGTATGGAGGGTCTAGCCTGGGTGAGTATGGAGGGTCTAGCCTGGGTG
TGTATGGAGGGTCTAGCCTGGGTGAGTATGGAGGGTCTAGCCTGGGTGTGTATGGAGGGTCTAGCCTGGGT
GAGTATGGAGGGTCTAGCCTGGGTGTGTACGGAGGGTCTAGTCTGAGTGCGTGTGGGGACCTCAGAACACT
GTGACCTTAGCCCCAGCAAGCCAGGCCCTTCAAGAAGGCCAAGAAGGCTGCCACCATTCCCTGCCAGCCCCAAGAA
CTCCAGCTTCCCCACTGCCTCTGTGTGCCCCCTTTCGCTCCTGTGAAGGCCATTGAGAAATGCCAGTGTGC
CCCCGAGGAAAGGGCACGGCCTGTGCTCCTGACACGGGCTGTGCTTGGCCACAGAACCCAGCGTCTCC
CCTGCTGTGTCCACGTCACTTCATGAGGCAACGTGCGGTGGTCTCAGACGTGGAGCAGCCAGCGGCAGCT
CAGAGCAGGGCACTGTGTCCGGCGGAGCCAAGTCCACTCTGGGGAGCTCTGGCGGGGACCACGGGCCACT
GCTACCCACTGGCCCCGAGGGGGGTGTAGACGCCAAGACTCACGCATGTGTGACATCCGGAGTCTCTGGAG
CCGGGTGTCCAGTGGCACCCTAGGTGCCCTGTGCTGCCCTCCACAGTGGGGTTCACACCCAGGGCTCCTTGGT
CCCCACAACCTGCCCCGGCCAGGCCTGCAGACCCAGACTCCAGCCAGACCTGCCTACCCACCAATGCAG
CCGGGGCTGGCGACACCAGCCAGGTGCTGGTCTTGGGCCAGTTCTCCACGACGGCTCACCTCCCCCTCCA
TCTGCGTTGATGCTCAGAATCGCCTACCTGTGCCTGCGTGTAAACCACAGCCTCAGACCAGCTATGGGGAG
AGGACAACACGGAGGATATCCAGCTTCCCGGTCTGGGGTGAGGAATGTGGGGAGCTTGGGCATCCTCCTC
CAGCCTCCTCAGCCCCCAGGCAGTGCCCTTACCTGTGGTGCCAGAAAAGTGCCCCCTAGGTTGGTGGGTCT
ACAGGAGCCTCAGCCAGGCAGCCACCCACCCCTGGGGCCCTGCCTACCAAGGAAATAAAGACTCAAGCC
ATAAAAAAA

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FIGURE 18

MRPGAPGPLWPLPWGALAWAVGFVSSMSGGNPAPGGVCWLQQGQEATCSLVLQTDVTRAECASGNIDTAW
SNLTHPGNKINLLGFLGLVHCLPCKDSCDGVCEGPGKACRMLGGRPRCECAPDCSGLPARLQVCGSDGATY
RDECELRAARCRGHPDLSVMYRGRCRKSCHEVVCPRPQSCVVDQTGSAHCVVCRAAPCPVPSSPGQELCGN
NNVTYISSCHMRQATCFLGRSIGVRHAGSCAGTPEEPPGGESAEEEENFV

Signal peptide:

Amino acids 1-20

N-glycosylation sites:

Amino acids 73-77;215-219

Osteonectin domain proteins:

Amino acids 97-130;169-202

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FIGURE 19

GAGCAGTGTCTGCTGGAGCCGATGCCAAAAACCATGCATTTCTTATTCAGATTCATTGTTTTCTTTTATC
TGTGGGGCCCTTTTACTGCTCAGAGACAAAAGAAAGAGGAGAGCACCGAAGAAGTGAAAAATAGAAGTTTTG
CATCGTCCAGAAAACTGCTCTAAGACAAGCAAGAAGGGAGACCTACTAAATGCCATTATGACGGCTACCT
GGCTAAAGACGGCTCGAAATTCTACTGCAGCCGGACACAAAATGAAGGCCACCCCAAATGGTTTGTCTTG
GTGTTGGGCAAGTCATAAAAGGCCTAGACATTGCTATGACAGATATGTGCCCTGGAGAAAAGCGAAAAGTA
GTTATACCCCTTCATTTGCATACGGAAGGAAGGCTATGCAGAAGGCAAGATTCCACCGGATGCTACATT
GATTTTTGAGATTGAACCTTTATGCTGTGACCAAAGGACCACGGAGCATTGAGACATTTAAACAAATAGACA
TGGACAATGACAGGCAGCTCTCTAAAGCCGAGATAAACCTCTACTTGCAAAGGGAATTTGAAAAAGATGAG
AAGCCACGTGACAAGTCATATCAGGATGCAGTTTTAGAAAGATATTTTAAGAAGAATGACCATGATGGTGA
TGGCTTCATTTCTCCCAAGGAATACAATGTATACCAACACGATGAACTATAGCATATTTGTATTTCTACTT
TTTTTTTTTAGCTATTTACTGTACTTTATGTATAAAACAAAGTCATTTTCTCCAAGTTGTATTTGCTATT
TTCCCTATGAGAAGATATTTTGATCTCCCAATACATTGATTTTGGTATAATAAATGTGAGGCTGTTTT
GCAAACTTAAA

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FIGURE 20

MPKTMHFLFRFIVFFYLWGLFTAQRQKKEESTEEVKIEVLHRPENCSKTSKKGDLLNAHYDGYLAKDGSKF
YCSRTQNEGHPKWFVLGVGQVIKGLDIAMTDMCPGEKRKVVIPPSFAYGKEGYAEGKIPPDATLIFEIELY
AVTKGPRSIETFKQIDMDNDRQLSKAEINLYLQREFEKDEKPRDKSYQDAVLEDIFKKNHDHGDGDFISPKE
YNVYQHDEL

Endoplasmic reticulum targeting sequence:	Amino acids 219-224
N-glycosylation site:	Amino acids 45-49
FKBP-type peptidyl-prolyl cis-trans isomerase:	Amino acids 87-124; 129-143
EF-hand calcium-binding domain proteins:	Amino acids 202-215; 195-215
Tyrosine kinase phosphorylation site:	Amino acids 183-190
N-myristoylation sites:	Amino acids 19-25; 95-101

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FIGURE 21

CCCACGCGTCCGCGCAGTCGCGCAGTTCTGCCTCCGCCTGCCAGTCTCGCCCGCGATCCCGGCCCGGGGCT
GTGGCGTCGACTCCGACCCAGGCAGCCAGCAGCCCGCGCGGGAGCCGGACCGCCCGCGGAGGAGCTCGGAC
GGCATGCTGAGCCCCCTCCTTTGCTGAAGCCCGAGTGCGGAGAAGCCCGGGCAAACGCAGGCTAAGGAGAC
CAAAGCGGCGAAGTCGCGAGACAGCGGACAAGCAGCGGAGGAGAAGGAGGAGGAGGCGAACCCAGAGAGGG
GCAGCAAAGAAGCGGTGGTGGTGGGCGTCGTGGCCATGGCGGCGGGCTATCGCCAGCTCGCTCATCCGTCA
GAAGAGGCAAGCCCGCGAGCGCGAGAAATCCAACGCCTGCAAGTGTGTGTCAGCAGCCCCAGCAAAGGCAAGA
CCAGCTGCGACAAAAACAAGTTAAATGTCTTTTCCCGGGTCAAACCTCTTCGGCTCCAAGAAGAGGCGCAGA
AGAAGACCAGAGCCTCAGCTTAAGGGTATAGTTACCAAGCTATACAGCCGACAAGGCTACCACTTGCAGCT
GCAGGCGGATGGAACCATTTGATGGCACCAAGATGAGGACAGCACTTACACTCTGTTAACCTCATCCCTG
TGGGTCTGCGAGTGGTGGCTATCCAAGGAGTTCAAACCAAGCTGTACTTGGCAATGAACAGTGAGGGATAC
TTGTACACCTCGGAACTTTTCACACCTGAGTGCAAATCAAAGAATCAGTGTTTGAAAATTATTATGTGAC
ATATTCATCAATGATATACCGTCAGCAGCAGTCAGGCCGAGGGTGGTATCTGGGTCTGAACAAAGAAGGAG
AGATCATGAAAGGCAACCATGTGAAGAAGAACAAGCCTGCAGCTCATTTTCTGCCTAAACCACTGAAAGTG
GCCATGTACAAGGAGCCATCACTGCACGATCTCACGGAGTTCTCCCGATCTGGAAGCGGGACCCCAACCAA
GAGCAGAAGTGTCTCTGGCGTGCTGAACGGAGGCAAATCCATGAGCCACAATGAATCAACGTAGCCAGTGA
GGGCAAAGAAGGGCTCTGTAACAGAACCCTTACCTCCAGGTGCTGTTGAATTCTTCTAGCAGTCCTTCACC
CAAAGTTCAAATTTGTCAGTGACATTTACCAAACAAACAGGCAGAGTCACTATTCTATCTGCCATTAGA
CCTTCTTATCATCCATACTAAAGC

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FIGURE 22

MAAAIAS\$LIROKQAREREKSNACKCVSSPSKGKTSCKDNKLNVSFVKLFGSKKRRRRRPEPQLKGIVT
KLYSRQGYHLQLQADGTIDGTDKEDSTYTLFNLIPVGLRVVAIQGVQTKLYLAMNSEGYLYTSELFTPECK
FKESVFENYYVTYSSMIYRQQQSGRGWYLGLNKEGEIMKGNHVKKNKPAAHFLPKPLKVAMYKEPSLHDLT
EFSRSGSGTPTKSRSVSGVLNGGKSMHNEST

N-glycosylation site:	Amino acids 242-246
Glycosaminoglycan attachment sites:	Amino acids 165-169, 218-222
Tyrosine kinase phosphorylation site:	Amino acids 93-100
N-myristoylation sites:	Amino acids 87-93, 231-237
ATP/GTP-binding site motif A (P-loop):	Amino acids 231-239
HBGF/FGF family proteins:	Amino acids 78-94, 102-153

FIGURE 23

CAGCGCTGACTGCGCCGCGGAGAAAGCCAGTGGGAACCCAGACCCATAGGAGACCCGCGTCCCCGCTCGGC
CTGGCCAGGCCCCGCGCTATGGAGTTCTCTGGGCCCTCTCTTGGGTCTGTGCTGCAGTCTGGCCGCTGC
TGATCGCCACACCGTCTTCTGGAACAGTTCAAATCCCAAGTTCCGGAATGAGGACTACACCATACATGTGC
AGCTGAATGACTACGTGGACATCATCTGTCCGCACTATGAAGATCACTCTGTGGCAGACGCTGCCATGGAG
CAGTACATACTGTACCTGGTGGAGCATGAGGAGTACCAGCTGTGCCAGCCCCAGTCCAAGGACCAAGTCCG
CTGGCAGTGCAACCGGCCCAGTGCCAAGCATGGCCCCGAGAAGCTGTCTGAGAAGTTCCAGCGCTTCACAC
CTTTACCCCTGGGCAAGGAGTTCAAAGAAGGACACAGCTACTACTACATCTCCAAACCCATCCACCAGCAT
GAAGACCGTGCTTGAGGTTGAAGGTGACTGTGCTGAGTGGCAAAATCACTCACAGTCCCTCAGGCCCCATGACAAT
CCACAGGAGAAGAGACTTGCAGCAGATGACCCAGAGGTGCGGGTTCTACATAGCATCGGTACAGTGCTGC
CCCACGCCCTCTTCCCACTTGCCCTGGACTGTGCTGCTCCTTCCACTTCTGCTGCTGCAAACCCCGTGAAGGT
GTGTGCCACACCTGGCCTTAAAGAGGGACAGGCTGAAGAGAGGGACAGGCACTCCAAACCTGTCTTGGGGC
CACTTTTCAAGAGCCCCAGCCCTGGGAACCACTCCACCACAGGCATAAGCTATCACCTAGCAGCCTCAAAA
CGGGTCAATATTAAGGTTTTCAACCGGAAGGAGGCCAACCAGCCCGACAGTGCCATCCCCACCTTCACCTC
GGAGGGATGGAGAAAGAAGTGGAGACAGTCCCTTCCACCATTCTGCTTTAAGCCAAAGAAACAAGCTG
TGCAGGCATGGTCCCTTAAGGCACAGTGGGAGCTGAGCTGGAAGGGGCCACGTGGATGGGCAAAGCTTGT
AAAGATGCCCCCTTCAAGGAGAGAGCCAGGATGCCAGATGAACTGACTGAAGGAAAAGCAAGAAACAGTTT
CTTGCTTGAAGCCAGGTACAGGAGAGGCAGCATGCTTGGGCTGACCCAGCATCTCCAGCAAGACCTCAT
CTGTGGAGCTGCCACAGAGAAGTTTGTAGCCAGGTAAGTCTCTCCCATCCTGGGGCAGCACTCCCCA
GAGCTGTGCCAGCAGGGGGCTGTGCCAACCTGTTCTTAGAGTGTAGCTGAAGGGCAGTGCCCATGTGTA
CATTCTGCCTAGAGTGTAGCCTAAAGGGCAGGGCCCACGTGTATAGTATCTGTATATAAGTTGCTGTGTGT
CTGTCTGATTTCTACAACCTGGAGTTTTTTTATACAATGTTCTTGTCTCAAATAAAGCAATGTGTTTTT
TCGG

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FIGURE 24

MEFLWAPLLGLCCSLAAADRHTVFWNSSNPKFRNEDYTIHVQLNDYVDIICPHYEDHSVADAAMEQYILYL
VEHEEYQLCQPQSKDQVRWQCNRPQSAKHGPEKLSEKFQRTFPFTLGKEFKEGHSYYYISKPIHQHEDRCLR
LKVTVSGKITHSPQAHDPQEKRLAADDPEVRVLHSIGHSAAPRLFPLAWTVLLLPLLLLQTP

Signal sequence: Amino acids 1-17

N-glycosylation site: Amino acids 26-30

Tyrosine kinase phosphorylation site:
Amino acids 118-127

N-myristoylation site: Amino acids 10-16

FIGURE 25

CGCCGCCCCGCGCCTGCCTGGGCCGGGGCCGAGGATGCGGCGCAGCGCCTCGGGCGGCCAGGGCTCGCTCCCCCT
CCGGCACGCCTGCTAACTTCCCCCGCTACGTCCCCGTTTCGCCCGCCGGGGCCGCCCGCTCTCCCCGCGCCCT
CCGGGTGCGGTCTCTCCAGGAGCGCCAGGCGCTGCCGCCGTGTGCCCTCCGCCGCTCGCCCCGCGCGCCGCG
CTCCCCGCTGCGCCCAGCGCCCCGCGCCCCGCGCCAGTCTCGGGCGGTCATGCTGCCCTCTGCCTCGT
GGCCGCCCTGCTGCTGGCCGCGGGGCCGGGGCCGAGCCTGGGCGACGAAGCCATCCACTGCCCGCCCTGCT
CCGAGGAGAAGCTGGCGCGCTGCCGCCCCCCCCGTGGGCTGCGAGGAGCTGGTGCAGAGAGCCGGGCTGCGGC
TGTTGCGCCACTTGCGCCCTGGGCTTGGGGATGCCCTGCCGGGGTGACACCCCCCGTTGCGGCTCGGGCCT
GCGCTGCTACCCGCCCCGAGGGGTGGAGAAGCCCCCTGCACACACTGATGCACGGGCAAGGCGTGTGCATGG
AGCTGGCGGAGATCGAGGCCATCCAGGAAAGCCTGCAGCCCTCTGACAAGGACGAGGGTGACCACCCCAAC
AACAGCTTCAGCCCCCTGTAGCGCCCATGACCGCAGGTGCCTGCAGAAGCACTTCGCCAAAATTTCGAGACCG
GAGCACCAAGTGGGGGCAAGATGAAGGTCAATGGGGCGCCCCGGGAGGATGCCCGCCTGTGCCCCAGGGCT
CCTGCCAGAGCGAGCTGCACCGGGCGCTGGAGCGGCTGGCCGCTTCACAGAGCCGCACCCACGAGGACCTC
TACATCATCCCCATCCCCAACTGCGACCGCAACGCAACTTCACCCCAAGCAGTGTACCCAGCTCTGGAT
GGGCAGCGTGGCAAGTGCTGGTGTGTGGACCGGAAGACGGGGGTGAAGCTTCGCGGGGGCTGGAGCCAAA
GGGGGAGCTGGACTGCCACCAGCTGGCTGACAGCTTTCGAGAGTGAGGCCTGCCAGCAGGCCAGGGACTCA
GCGTCCCCCTGCTACTCCTGTGCTCTGGAGGCTGCAGAGCTGACCCAGAGTGGAGTCTGAGTCTGAGTCTCTG
TCTCTGCCTGCGGCCAGAAGTTTCCCTCAAATGCGCGTGTGCACGTGTGCGTGTGCGTGTGCTGTGTGTGT
GTTTGTGAGCATGGGTGTGCCCTTGGGGTAAGCCAGAGCCTGGGGTGTTCTCTTTGGTGTTACACAGCCCA
AGAGGACTGAGACTGGCACTTAGCCCCAAGAGGTCTGAGCCCTGGTGTGTTTCCAGATCGATCCTGGATTCA
CTCACTCACTCATTCCCTCACTCATCCAGCCACCTAAAAACATTTACTGACCATGTACTACGTGCCAGCTC
TAGTTTTACGCCCTTGGGAGGTTTTATTCTGACTTCCCTGATTTTGGCATGTGGAGACACTCCTATAAGGA
GAGTTCAAGCCTGTGGGAGTAGAAAAATCTCATTCCAGAGTCAGAGGAGAAGAGACATGTACCTTGACCA
TCGTCTTCTCTCAAGCTAGCCAGAGGGTGGGAGCCTAAGGAAGCGTGGGGTAGCAGATGGAGTAATGGT
CACGAGGTCCAGACCCACTCCCAAAGCTCAGACTTGCCAGGCTCCCTTTCTTCTTCCCCAGGTCTTCC
TTTAGGTCTGGTTGTGCACCATCTGCTTGGTTGGCTGGCAGCTGAGAGCCCTGCTGTGGGAGAGCGAAGG
GGGTCAAAGGAAGACTTGAAGCACAGAGGGCTAGGGAGGTGGGGTACATTTCTCTGAGCAGTCAGGGTGGG
AAGAAAGAATGCAAGAGTGGACTGAATGTGCCTAATGGAGAAGACCCACGTGCTAGGGGATGAGGGGCTTC
CTGGGTCTGTTCCTACCCCATTTGTGGTCACAGCCATGAAGTCACCGGGATGAACCTATCCTTCCAGTG
GCTCGCTCCCTGTAGCTCTGCCTCCCTCTCCATATCTCCTTCCCCTACACCTCCCTCCCCACACCTCCCTA
CTCCCCTGGGCATCTTCTGGCTTGACTGGATGGAAGGAGACTTAGGAACCTACCAGTTGGCCATGATGTCT
TTTCTTCTTTTCTTTTAAACAAAACAGAACAAAACCAAAAAATGTCCAAA

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FIGURE 26

MLPLCLVAALLLAAGPGPSLGDEAIHCPPCSEKILARCRPPVGCEELVREPGCGCCATCALGLGMPCGVYT
PRCGSGLRCYPPRGVEKPLHTLMHGQGVCMELAEIEAIQESLQPSDKDEGDHPNNSFSPCSAHDRRCLOKH
FAKIRDRSTSGGKMKVNGAPREDARPVPOGSCQSELHRALERLAASQSRTHEDLYIIPNCDRNGNFHPK
QCHPALDQGQKGCWCVDRKTGVKLPGGLEPKGELDCHQLADSFRE

Signal sequence: Amino acids 1-21
transmembrane domain: Amino acids 51-69 (weak)
N-glycosylation site: Amino acids 125-129
Tyrosine kinase phosphorylation site: Amino acids 191-198
N-myristoylation sites: Amino acids 52-58; 54-60; 64-70; 96-102; 172-178
Insulin-like growth factor binding proteins signature: Amino acids 52-68
IGF binding protein (less stringent than prosite's): Amino acids 52-61

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FIGURE 27

AGCTCCAGCTGACCACCGGAATGGCCTGTTCCCTCAGCGAGGGCCTGAAGCTCCCACCAGGCCAGCTCTCCT
CCCACAACAGCTTCCCACAGCATGAAGATCTCCGTGGCTGCCATTCCCTTCTTCCTCCTCATCACCATCGC
CCTAGGGACCAAGACTGAATCCTCCTCACGGGGACCTTACCACCCCTCAGAGTGCTGCTTCACCTACACTA
CCTACAAGATCCCGCGTCAGCGGATTATGGATTACTATGAGACCAACAGCCAGTGCTCCAAGCCCGGAATT
GTCTTCATCACCAAAAGGGGCCATTCCGTCTGTACCAACCCAGTGACAAGTGGGTCCAGGACTATATCAAG
GACATGAAGGAGAACTGAGTGACCCAGAAGGGGTGGCGAAGGCACAGCTCAGAGACATAAAGAGAAGATGC
CAAGGCCCCCTCCTCCACCCACCGCTAACTCTCAGCCCCAGTCACCCTCTT

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FIGURE 28

MKISVAAIPFFLLITIALGKTESSSRGPYHPSECCFTYTTYKIPQRIMDYETNSQCSKPGIVFITKRG
HSVCTNPSDKWVQDYIKDMKEN

Signal sequence:

Amino acids 1-22

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FIGURE 29

CCCACAGGAGGAATGAAGGTCTCCGTGGCTGCCCTCTCCTGCCTCATGCTTGTTGCTGTCCTTGGATCCCAG
GCCCAGTTCACAAATGATGCAGAGACAGAGTTAATGATGTCAAAGCTTCCACTGGAAAAATCCAGTAGTTCT
GAACAGCTTTCACCTTGCTGCTGACTGCTGCACCTCCTACATCTCACAAAGCATCCCGTGTTCACTCATGA
AAAGTTATTTTGAACGAGCAGCGAGTGCTCCAAGCCAGGTGTCATCTTCCTCACCAAGAAGGGGCGGCAA
GTCTGTGCCAAACCCAGTGGTCCGGGAGTTCAGGATTGCATGAAAAAGCTGAAGCCCTACTCAATATAATA
ATAAAGAGACAAAGAGGCCAGCCACCCACCTCCAACACCTCCTAGATCTGATC

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FIGURE 30

MKVSVAALSCLMLVAVLGSQAQFTNDAETELMMSKLPLENPVVLNSFHFAADCCTSYISQSIPCSLMKSYF
ETSSECSKPGVIFLTKKGRQVCAKPSGPGVQDCMKKLPYSI

Signal sequence:	Amino acids 1-21
Glycosaminoglycan attachment site:	Amino acids 97-101
N-myristoylation site:	Amino acids 100-106

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FIGURE 31

GTGAAGGGAGCCGGGATCAGCCAGGGGCCAGCATGAGCCGGAGGGAGGGAAGTCTGGAAGACCCCCAGACT
GATTCCTCAGTCTCACTTCTTCCCCACTTGGAGGCCAAGATCCGTGAGACACACAGCCTTGCGCACCTCCT
CACCAAATACGCTGAGCAGCTGCTCCAGGAATATGTGCAGCTCCAGGGAGACCCCTTCGGGCTGCCCAGCT
TCTCGCCGCCGCGGCTGCCGGTGGCCGGCCTGAGCGCCCCGGCTCCGAGCCACGCGGGGCTGCCAGTGCAC
GAGCGCTGCGGCTGGACGCGGCGGCGCTGGCCGCGCTGCCCCGCTGCTGGACGCAGTGTGTGCGCCCA
GGCCGAGCTGAACCCGCGCGCGCGCGCCTGCTGCGCCGCTGGAGGACGCGGCGCGCCAGGCCCGGGCCC
TGGGCGCCGCGCTGGAGGCCCTTGCTGGCCGCGCTGGGCGCCGCCAACCGCGGGCCCCGGGCCGAGCCCCC
GCCGCCACCGCCTCAGCCGCTCCGCCACCGGGTCTTCCCCGCCAAGTGCTGGGGCTCCGCGTTTGCGG
CCTCTACCGGAGTGGCTGAGCCGCACCGAGGGCGACCTGGGCCAGCTGCTGCCCGGGGGCTCGGCCTGAG
CGCCGCGGGGAGCTCGCCCCGCTCCTCCCGCTGGGTTCGCTCTCTCCTTCCGCTTCTTTGTCTTCTCT
GCCGCTGTCGGTGTCTGTCTGTCTGCTCTTAGCTGTCTCATTGCCTCGGCCTTCTTTGCTTTTGTGGGG
GAGAGGGGAGGGGACGGGCAGGGTCTCTGTGCGCCAGGCTGGGGTGCACTGGCGCGATCCAGCACTGCAG
CCTCAACCTCCTGGGCTCAAGCCATCCTTCCGCTCAGCTTCCCCAGCAGCTGGGACTACAGGCACGCGCC
ACCACAGCCGGCTAATTTTTTATTTAATTTTTTGTAGAGACGAGGTTTCGCCATGTTGCCCAGGCTGGTCT
TGAATCCGGGGCTCAAGCGATCCTCCCGCTTCAGCCTCCCTAAGTGCTGGGATTGCAGGCGTGAGCCACT
TTCCAGCCTCTCTTTGCTTTGCCTGCCCCGTTCTCTTAACCTCTGGACCCTCCTCGTCTGCATGGTAACT
CCGTCTGAGTCTACCATTTTCTTGCTCTCCCTCCTTCTTGGGCCTGCCTCAGTTCCTTTGGCCTCCCCC
TTTACCAGCTCTTGGGGTGTCTCTGTTTTTCCATCCCCACTTCCTGCCTTCTCGTGGCCCTGTGTGAGC
ACATGTGTACATCTCAGCCTTATCTCAAGGAGGTGACACCTTCTCTCCTTGTCCCCATCTGGCCGTCTCTCTGT
GCTTCCCTGGCCAGGGCGTGCTGCTGGTCCATATGGGGGAAGGCTACTCCGCATCTCAGCCACCTTCCT
CAGGCTCACTCCACCTACATCCCCAGTCTGCCACACCCATCCCTTTGGGCCTCAGCCCTGTCCCTTTGAT
GTCCTCCTTTCTTCAGCCCTCTGCCCTGTCCCTGCACACCTCC

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FIGURE 32

MSRREGSLEDPQTDSSVSLLPHLEAKIRQTHSLAHLTKYAEQLLQEYVQLQGDPFGLPSFSPPRLPVAGL
SAPAPSHAGLPVHERLRRLDAAALAALPPLLDVCCRQAELNPRAPRLRRLEDAARQARALGAAVEALLAA
LGAANRGFRAEPPAATASAASATGVFPAKVLGLRVCGLYREWLSRTEGDLGQLLPGGSA

N-myristoylation sites:

Amino acids 166-172;174-180

Leucine zipper pattern:

Amino acids 37-59

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FIGURE 33

CCCAGCGCACCCGCACCAATGGCCGGCCCCAGCCTCGCTTGCTGTCTGCTCGGCCTCCTGGCGCTGACCTCC
GCCTGCTACATCCAGAAGTGGCCCCCTGGGAGGCAAGAGGGCCGCGCCGGACCTCGACGTGCGCAAGTGCCT
CCCCTGCGGCCCCGGGGGCAAAGGCCGCTGCTTCGGGCCCCAATATCTGCTGCGCGGAAGAGCTGGGCTGCT
TCGTGGGCACCGCCGAAGCGCTGCGCTGCCAGGAGGAGAACTACCTGCCGTGCGCCCTGCCAGTCCGGCCAG
AAGGCGTGCGGGAGCGGGGGCCGCTGCGCGGTCTTGGGCCTCTGCTGCAGCCCGGACGGCTGCCACGCCGA
CCCTGCCTGCGACGCGGAAGCCACCTTCTCCAGCGCTGAAACTTGATGGCTCCGAACACCCTCGAAGCGC
GCCACTCGCTTCCCCATAGCCACCCAGAAATGGTGAAAATAAAATAAAGCAGGTTTTTCTCCTCT

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FIGURE 34

Mature oxytocin:

CYIQNCPLGGKRAAPDLVVRKCLPCGPGGKGRCFGPNICCAEELGCFVGTAEALRCQEENYLPSPCQSGQK
ACGSGGRC AVLGLCCSPDGCHADPACDAEATFSQR

Pro-oxytocin:

MAGPSLACCLLGLLALTSACYIQNCPLGGKRAAPDLVVRKCLPCGPGGKGRCFGPNICCAEELGCFVGTAE
ALRCQEENYLPSPCQSGQKACGSGGRC AVLGLCCSPDGCHADPACDAEATFSQR

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| PCT/US99/20111 | | |
| | 1 September 1999 (01.09.1999) | US |
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| | 30 November 1999 (30.11.1999) | US |
| PCT/US99/28409 | | |
| | 30 November 1999 (30.11.1999) | US |
| PCT/US99/28565 | | |
| | 2 December 1999 (02.12.1999) | US |
| PCT/US99/30095 | | |
| | 16 December 1999 (16.12.1999) | US |
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| PCT/US00/04341 | | |
| | 18 February 2000 (18.02.2000) | US |
| PCT/US00/04342 | | |
| | 18 February 2000 (18.02.2000) | US |
| PCT/US00/05004 | | |
| | 24 February 2000 (24.02.2000) | US |
| PCT/US00/05841 | 2 March 2000 (02.03.2000) | US |
| PCT/US00/06319 | 10 March 2000 (10.03.2000) | US |
| PCT/US00/06884 | 15 March 2000 (15.03.2000) | US |
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[Continued on next page]

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(54) Title: INTERLEUKIN-1-RECEPTOR ASSOCIATED KINASE-3 (IRAK3)

(57) Abstract: Compositions and methods are disclosed for stimulating or inhibiting angiogenesis and/or cardiovascularization in mammals, including humans. Pharmaceutical compositions are based on polypeptides or antagonists thereto that have been identified for one or more of these uses. Disorders that can be diagnosed, prevented, or treated by the compositions herein include trauma such as wounds, various cancers, and disorders of the vessels including atherosclerosis and cardiac hypertrophy. In addition, the present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.



(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/13705

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/47 C07K14/52 A61K38/17 C07K16/18
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 25834 A (GENENTECH INC) 27 May 1999 (1999-05-27) the whole document	1-24, 29, 31-34, 38, 40-48, 59-80
X	WO 98 07880 A (HUMAN GENOME SCIENCES INC ; NI JIAN (US); ROSEN CRAIG A (US); YU GU) 26 February 1998 (1998-02-26) the whole document	1-4, 11-24, 47, 48, 59-80

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 00/13705

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GURNEY A L ET AL: "Identification of a new member of the tumor necrosis factor family and its receptor, a human ortholog of mouse GITR" CURRENT BIOLOGY,CURRENT SCIENCE,,GB, vol. 9, no. 4, 25 February 1999 (1999-02-25), pages 215-218, XP000938588 ISSN: 0960-9822 the whole document	1-4,6, 11-24, 59-80
X	--- KWON BYUNGSUK ET AL: "Identification of a novel activation-inducible protein of the tumor necrosis factor receptor superfamily and its ligand." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 10, 5 March 1999 (1999-03-05), pages 6056-6061, XP002147323 ISSN: 0021-9258 the whole document	1-4, 11-24, 47,48, 59-80
A	--- KLAGSBRUN M: "REGULATORS OF ANGIOGENESIS: STIMULATORS, INHIBITORS, AND EXTRACELLULAR MATRIX" JOURNAL OF CELLULAR BIOCHEMISTRY,US,LISS, NEW YORK, NY, vol. 47, no. 3, 1 November 1991 (1991-11-01), pages 199-200, XP000616588 ISSN: 0730-2312 the whole document	
A	--- WO 95 29242 A (CHILDRENS MEDICAL CENTER) 2 November 1995 (1995-11-02) the whole document	
A	--- EP 0 335 243 A (HARVARD COLLEGE) 4 October 1989 (1989-10-04) the whole document	
P,X	--- WO 99 40196 A (GENENTECH INC) 12 August 1999 (1999-08-12) examples 2,10,11 -----	1,2,6,7, 10,11, 13-15, 17,18, 31-34, 38,42, 45-48, 59-65, 67, 70-73, 79,80

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/13705

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 31-45 and 49-58 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-49, 52, 59-80

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: Invention 1: claims 1-49,52,59-80, all partially

Composition comprising PR0175 polypeptide or (ant)agonist thereof, fusion proteins comprising a portion of said protein, antibody against said protein, method for identifying (ant)agonists of said protein or compounds which modulate the expression level or the activity of said protein, methods for modulating the activity of said protein using said (ant)agonists or modulators, methods for diagnosis comprising assessing the protein or expression levels of said polypeptide, retroviral particle encoding said protein or (ant)agonist, and producer cell of said protein or (ant)agonist comprising said retroviral particle, use of said protein, (ant)agonists thereof or nucleic acids encoding said protein in the manufacture of a composition for the treatment of angiogenesis or cardiovascularization related disorder.

2. Claims: Inventions 2-17: claims 1-80,
all partially and in as far as applicable

Idem as subject 1 but limited to the respective proteins PR0200, PR0198, PR0364, PR0356, PR0535, PR0819, PR01002, PR01308, PR01304, PR0183, PR0202, PR0861, PR0877, PR0879, PR0882, and PR01710.

For the sake of conciseness, the first subject matter is explicitly defined, the other subject matters are defined by analogy thereto.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

A meaningful search for the full scope of claims 1,2,5-7,10,17-19,31-35,38,42, and 45-54, referring to (ant)agonists of the claimed proteins and compounds inhibiting the expression thereof, could not be performed due to insufficient characterization of the (ant)agonists and/or expression inhibitors in the description (lack of disclosure, support, clarity, and conciseness, PCT articles 5 & 6). It is clear from e.g. claims 3,4 and 20, however, that the claimed (ant)agonists could be agonistic antibodies against proteins defined in the application, and that the expression inhibiting compound could be an antisense molecule. The search for the above claims, in as far as they relate to (ant)agonists and expression inhibitors, was therefore limited to (ant)agonistic antibodies and antisense molecules only.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter. .nal Application No

PCT/US 00/13705

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9925834 A	27-05-1999	AU 1418099 A EP 1032672 A	07-06-1999 06-09-2000
WO 9807880 A	26-02-1998	AU 6847896 A EP 0961831 A	06-03-1998 08-12-1999
WO 9529242 A	02-11-1995	US 5639725 A US 5792845 A AU 692865 B AU 2461795 A BR 9507479 A CA 2188813 A CN 1149319 A CZ 9603122 A EP 0758390 A HU 76095 A JP 9512173 T NZ 285501 A US 5885795 A US 5733876 A US 5776704 A ZA 9503419 A	17-06-1997 11-08-1998 18-06-1998 16-11-1995 16-09-1997 02-11-1995 07-05-1997 14-05-1997 19-02-1997 30-06-1997 09-12-1997 26-02-1998 23-03-1999 31-03-1998 07-07-1998 11-01-1996
EP 0335243 A	04-10-1989	US 4900673 A AT 96844 T AU 621358 B AU 3433189 A CA 1331356 A DE 68910354 D DE 68910354 T DK 233390 A JP 3503641 T PT 90129 A WO 8909277 A ZA 8902270 A	13-02-1990 15-11-1993 12-03-1992 16-10-1989 09-08-1994 09-12-1993 03-03-1994 26-11-1990 15-08-1991 10-11-1989 05-10-1989 24-12-1991
WO 9940196 A	12-08-1999	AU 2591599 A EP 1053321 A	23-08-1999 22-11-2000